Wildlife Health Program
Wildlife Populations and Research Group
5463 - C West Broadway
Forest Lake, Minnesota 55025
(651) 296-5200

SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WILD WATERFOWL IN 2015

Chris Jennelle[1], Michelle Carstensen, Erik Hildebrand, and Lou Cornicelli

SUMMARY OF FINDINGS

Since detection of highly pathogenic avian influenza (HPAI) strain H5N2 in a poultry facility in Pope County MN on February 27 2015, the Minnesota Department of Natural Resources (MNDNR) partnered with the United States Department of Agriculture's Wildlife Services (USDA-WS), the United States Geological Survey's National Wildlife Health Center (USGS), the United States Fish and Wildlife Service (USFWS), and the University of Georgia – Southeast Cooperative Wildlife Disease Study (SCWDS) to conduct surveillance for the virus in Minnesota waterfowl. From March through June 2015, 108 poultry facilities in MN tested positive for HPAI, resulting in severe economic losses to producers and local economic disruption. From March through August 2015, the MNDNR collected and tested 3,138 waterfowl fecal samples, 104 wild bird morbidity or mortality events, and 84 spring hunter-harvested wild turkeys (*Meleagris gallopavo*) for detectable HPAI. Only 1 HPAI positive case was confirmed on April 29 2015 from a wild bird mortality event; a Cooper's hawk (*Accipiter cooperii*). In addition, 100 waterfowl fecal and 2 wild bird mortality samples tested positive for low pathogenic avian influenza (LPAI). On July 10 2015, a black-capped chickadee (*Poecile atricapillus*) from Ramsey County, MN was submitted to the MN Wildlife Rehabilitation Center and also tested positive for HPAI. These two cases are the only verified HPAI positive wild birds detected in Minnesota through August 2015. In June and July 2015 the MNDNR collected 619 swab and blood samples of resident Canada geese (*Branta canadensis*) in central Minnesota. Only two swab samples indicated active shedding of LPAI, and results on serological analysis are pending at SCWDS. As part of our collaboration with SCWDS, the MNDNR is in the process of finishing collection of approximately 600 paired swab and blood samples from dabbling ducks in MN. There is concern from the poultry industry that fall migratory waterfowl movements will place poultry facilities in MN at greater risk of infection (or reinfection) and MNDNR is preparing additional HPAI surveillance efforts to test a sample of migratory waterfowl both in counties with poultry facilities that were infected in Spring 2015 and uninfected facilities. In addition to a MNDNR goal of collecting 800 tracheal and cloacal swab samples from fall hunter-harvested dabbling ducks, we will be collecting an additional 385 swab samples from dabbling ducks in fall and winter 2015 as part of a National USDA HPAI surveillance plan. Aside from these efforts, MNDNR will continue to monitor the health of wild birds by testing wild bird morbidity and mortality events, and screening for HPAI when appropriate.

[1] Corresponding author e-mail: christopher.jennelle@state.mn.us

INTRODUCTION

Avian Influenza (AI) is a viral infection that occurs naturally in wild birds, especially waterfowl, gulls, and shorebirds. It is caused by type A influenza viruses that have 2 important surface antigens, hemagglutinin (H) and neuraminidase (N), that give rise to 144 possible virus subtypes. Influenza viruses vary widely in pathogenicity and ability to spread among birds. The emergence of an Asian strain HPAI H5N1 virus in 1996 and subsequent spread of the virus in Asia, Africa, and Europe killed thousands of wild birds and millions of domestic poultry. In 1997, HPAI H5N1 became zoonotic in Hong Kong and to-date has infected at least 844 humans around the world, resulting in 449 deaths (World Health Organization 2015). Since there is a risk of worldwide pandemic due to quickly evolving strains of HPAI, there is an urgent need to understand transmission dynamics, host-species susceptibility, and role of the environment in AI dynamics.

From 2006 to 2010, the MNDNR tested over 12,000 wild birds in MN for HPAI and none were detected to be actively shedding virus (Hildebrand et al. 2010). The migratory movements of waterfowl and other shorebirds and subsequent mixing of birds from Asia and North America in the northern latitude breeding grounds facilitated the mixing of LPAI and HPAI strains. Such mixing has resulted in recent discovery of three reassortant highly pathogenic strains including H5N1 (World Organisation for Animal Health 2014), H5N2 (World Organisation for Animal Health 2014, Pasick et al. 2015), and H5N8 (Ip et al. 2015) in British Columbia and the western United States. On March 3 2015, a poultry facility in MN was confirmed infected with HPAI H5N2; the first time any HPAI strain has been detected in Minnesota poultry. The MNDNR subsequently conducted aerial surveillance for waterfowl in a 700 mi^2 surveillance zone centered on the infected farm. Only 100 mallards (*Anas platyrhynchos*) and 18 trumpeter swans (*Cygnus buccinator*) were detected, and staff collected 148 fresh feces samples from these individuals for AI testing. No HPAI was detected, but 6 LPAI positive samples were identified from the mallards, which demonstrated that the sampling technique was effective in detecting shed virus.

In late March, additional poultry facilities became infected with H5N2 and the MNDNR rapidly initiated surveillance efforts to detect the virus in wild birds and waterfowl feces. As such, our objectives were to conduct waterfowl feces, morbidity/mortality event, and hunter-harvested turkey sampling to detect H5N2 in wild birds and the environment (via feces) they inhabit. These efforts permit the estimation of HPAI distribution and magnitude on the Minnesota landscape, which leads to development of specific hypotheses that can help us understand and manage HPAI in wild birds. The scope of the outbreak in MN poultry facilities was unprecedented and by June 2015, 23 counties contained 108 infected farms (102 turkey, 5 chicken, and 1 backyard flock) and 9.3 million birds were euthanized (20% of MN's annual poultry population) causing an economic impact of 650 million dollars.

METHODS

Fecal, morbidity and mortality, and hunter-harvested surveillance

We collected three types of samples: waterfowl feces, public-reported morbid or dead wild birds (i.e., morbidity and mortality events), and hunter-harvested wild turkeys (*Meleagris gallopavo*). The sampling design differed for each data type and required different protocols for collecting samples. Given the rapid onset of infected poultry facilities, we focused primarily on collecting waterfowl feces for three reasons: (1) this sample type afforded us the most control over sampling design elements, (2) a large sample size could be collected relatively quickly (on the order of weeks), and (3) the timing of the outbreak coincided with breeding activities when birds do not congregate and available live capture methods are inefficient. The other two data types (sampling dead wild birds) depended on opportunistic circumstances and public willingness to report or submit dead birds.

We designed fecal sampling efforts to compare potential differences in H5N2 prevalence spatially. We choose five counties with infected facilities (Lac Qui Parle, Kandiyohi, Pope, Meeker, and Stearns) and five wildlife management areas (WMA)/National Wildlife Refuges (NWR) without infected facilities that typically attract large numbers of waterfowl (Carlos Avery WMA, Minnesota Valley NWR, Swan Lake WMA, Thief Lake WMA, and Whitewater WMA) (Figure 1). Since this was designed early in the Minnesota outbreak, we chose 5 of the initial counties that reported infected facilities as our treatment areas. Within the ten selected counties, we collated a list of available wetlands and lakes from which we scouted for waterfowl activity and sampled waterfowl feces deposited at 17 types of locations (Table 1) associated with waterfowl activity. We sampled only what we perceived to be fresh feces (<24 hrs old) that were at least two meters apart. We used polyester-tipped swabs to sample fecal material, placed samples in brain-heart infusion (BHI) medium, and stored media in a cooler with ice packs or a refrigerator. For any given site, swabs could be pooled in groups of 3 in the same media vial. For each county, our goal was to collect at least 300 fecal samples, and we

prioritized collecting no more than approximately 20 samples from a given location to obtain spatial representation within a target county. Assuming fecal samples represented individual waterfowl independently with test sensitivity and specificity of 100%, our lower bound on detection permitted at least a 95% probability of detecting H5N2 viral DNA if at least 1% of the population was actively shedding virus.

Through outreach on the MNDNR and Minnesota Board of Animal Health websites and official press releases, we solicited the public to report any wild birds exhibiting neurological symptoms consistent with AIV infection, dead raptors or wild turkeys, and groups of 5 or more dead birds of any species aggregate. We did not investigate reports of dead ducks as HPAI infected ducks are generally asymptomatic, and ducks that were confirmed with HPAI infection had died from other causes. However, we did investigate reports of dead Canada geese because recent evidence in Wyoming and Michigan documented HPAI H5N2 positive geese with clinical signs of illness. We emphasized the need to report dead birds as soon as possible to ensure collection of viable tissue samples; generally we only collected samples from birds that were deceased for <24 hours. Depending on the resources available for staff (e.g., BHI media), we either collected whole carcasses (double-bagged and frozen) or used swabs to sample tissues from the tracheal and cloacal cavities. Each swab sample was placed in the same BHI media, and kept cool in a portable cooler with ice packs or a refrigerator. Whole carcasses were sent overnight to the USGS for necropsy and AIV testing using RT-PCR. Swab samples were submitted to the USDA National Wildlife Disease Laboratory (Fort Collins, CO) for AIV testing using RT-PCR. If samples tested AIV positive initially at either lab, they were forwarded to the National Veterinary Services Laboratories (Ames, Iowa) for confirmation and strain-typing. We made no fixed sample goals for this sample type due to the opportunistic nature of public discovery and reporting. We used this data as an auxiliary source of information in our surveillance efforts, and obtained samples from all parts of Minnesota.

The H5N2 outbreak coincided with the spring harvest season for wild turkeys, and it is unclear whether this species is susceptible to infection. This afforded an opportunity for a pilot project, and we set a goal to collect swab samples from the tracheal and cloacal cavities of 300 hunter-harvested wild turkeys in the heart of affected poultry areas including Kandiyohi, Meeker, Pope, and Stearns counties. Hunter provision of harvested turkeys was voluntary and at sampling, swabs were placed in BHI media, and stored in a cooler with ice packs or a refrigerator. These samples were submitted to the National Wildlife Health Center (Madison, WI) for AIV testing using RT-PCR.

Paired serology and virus shedding surveillance: A SCWDS collaboration

The MNDNR is partnering with SCWDS in order to evaluate serological results of blood samples obtained from Canada geese and susceptible duck species (primarily mallard and blue-winged teal). Serology, although not yet established as a stand-alone surveillance tool, provides information about whether an animal has mounted an immune response to AIV and has circulating levels of antibodies present in its blood. There are several major limitations; one cannot discern when or how an animal was initially exposed to AIV, and subtyping HPAI and LPAI is an active area of research with unestablished standard protocols.

MNDNR established sampling sites for Canada geese in five area of Minnesota in counties that contained poultry facilities that experienced spring HPAI infection and counties with poultry facilities that were not affected (Figure 2). Sampling occurred during scheduled goose banding efforts with a special Bird Banding Laboratory code applied to records of geese with blood drawn. A goal of 3mL of blood was drawn from each sampled goose and was later centrifuged for serum, which was decanted into 2mL cryovials and stored frozen until shipment to SCWDS. We also collected oropharyngeal and cloacal swab tissue samples from each goose, pooled each pair of swabs in the same BHI media vial, and placed vials in a chilled cooler with ice packs for later transfer to a refrigerator for storage. These samples were submitted to the National Wildlife Health Center (Madison, WI) for AIV testing using RT-PCR.

Goose serum samples were shipped to SCWDS in late July, and are in the process of serological analysis.

Similarly, we selected six work areas for collecting paired samples (i.e., swabs and serology) from ducks (primarily mallards and teal) in counties that contained poultry facilities that experienced spring HPAI infection and counties with poultry facilities that were not affected (Figure 3). Sampling is currently underway with a goal of collecting 625 samples from all works areas. Each captured duck will receive a uniquely numbered aluminum leg band and ascribed a special Bird Banding Laboratory code indicating blood was drawn. A goal of 2mL of blood is drawn from each sampled goose and will be later centrifuged for serum, to be decanted into 2mL cryovials and stored frozen until shipment to SCWDS. We will also collect oropharyngeal and cloacal swab tissue samples from each duck, pool each pair of swabs in the same BHI media vial, and place vials in a chilled cooler with ice packs for later transfer to a refrigerator for storage. These samples will be submitted to the National Wildlife Health Center (Madison, WI) for AIV testing using RT-PCR. Duck serum samples will be shipped to SCWDS in late September of early October.

RESULTS AND DISCUSSION

From March 1 through August 31 2015, the MNDNR collected a combined total of 3,327 samples from the waterfowl feces (*n=3,138*), wild birds mortalities (*n=104*) (Table 2), and spring hunter-harvested wild turkeys (*n=84*) (Figure 4). Only one HPAI positive case was confirmed from a Cooper's hawk mortality sample in Yellow Medicine County on 29 April 2015; it was approximately 12.5 miles from the nearest infected poultry facility. This predatory bird is typically found in woodlands and has a diet consisting mainly of small birds and mammals, and we suspect the infected hawk was exposed to HPAI through a food item. Although not part of MNDNR surveillance, a black-capped chickadee submitted for testing from the MN Wildlife Rehabilitation Center was also confirmed positive on 10 July 2015. These two birds were the only confirmed HPAI infected wild bird samples in MN through 31 August 2015. A total of 100 fecal samples, 1 mortality sample (non-H5 or H7), and 0 wild turkey samples were determined to be LPAI positive. The testing protocol limited the screening for H5, H7, and N1 subtypes only; however, in some cases other subtypes were identified and reported elsewhere.

From the 619 Canada goose swabs samples collected, there were 0 HPAI positive and 2 LPAI positive cases based on RT-PCR analysis (Figure 5). Serology analysis of these samples is pending completion. The collection of paired swab-serology samples for dabbling ducks is currently underway with 196 paired samples collected on dabbling ducks. Data collection is expected to be complete by the end of September.

Future Surveillance

As part of the 2015 USDA National Surveillance Plan, MN is asked to submit 545 oropharyngeal and cloacal cavity swab samples of dabbling ducks for AIV testing to the USGS by the end of winter 2015 (Figure 6). The samples requests are broken down by watershed (Mississippi Headwaters, Red River, St.Croix, Upper Mississippi – Black Root, and Western Lake Superior) and season; summer, fall, and winter. The summer quota of 30 samples for the St. Croix and 130 samples for the Red River watersheds was achieved and is in the process of analysis at the USGS. The fall quota of 40 samples for the Red River, 140 samples for the Mississippi Headwaters, 50 samples for the St. Croix, 40 samples for the Upper Mississippi – Black Root, and 60 samples for the Western Lake Superior watersheds will be forthcoming. Similarly, the MNDNR expects to collect a winter quota of 55 samples from the Upper Mississippi – Black Root watershed.

MNDNR is continuing the collaboration with SCWDS to capture approximately 600 known HPAI susceptible ducks from August through September 2015. Paired tissue samples will be obtained from each duck captured; swab samples from the oropharyngeal and cloacal cavities and approximately 1.5ml of blood for serological testing. Again there are two types of

study areas at the county scale designated by whether poultry facilities experienced HPAI infection or not (Figure 3).

During the fall duck hunting season starting September 26 2015, MNDNR is planning to sample the tracheal and cloacal cavities of 800 hunter-harvested dabbling ducks. The sampling will take place in two types of study areas at the county scale designated by whether poultry facilities experienced HPAI infection or not (Figure 7). The sample size will be approximately split between the two types study areas for comparison. The sample size is chosen to detect HPAI viral shedding at a 1% prevalence level with at least 95% confidence assuming an 80% diagnostic test sensitivity and 100% specificity.

ACKNOWLEDGMENTS

These efforts would not have been possible without the valuable contribution of the Wetland Wildlife Population and Research Group, including J. Lawrence, B. Davis, P. Hagen, K. Young, AI techs M. Kuzel and S. McDowell, and banding interns. MNDNR management and research staff were invaluable in providing guidance for identifying sampling locations and capture/sampling assistance; they include K. Arola, C. August, R. Baden, F. Bengtson, T. Dale, M. Deters, M. Dexter, S. Gibbs, J. Guidice, J. Huener, J. Jaeger, A. Knutson, K. Kotts, W. Krueger, J. Labarre, B. Liddell, J. Markl, R. Markl, J. Miller, D. Nelson, C. Netland, M. Oehler, R. Olsen, B. Olson, K. Pharis, T. Pharis, S. Piepgras, B. Schuna, N. Snavely, J. Stangel, V. St-Louis, J. Strege, E. Thorson, T. Tonsager, N. Trauba, C. Vacek, J. Vorland, and A. Westmark. We recognize our USDA-WS partners P. Wolf, B. Welinski, and assistants; USFWS partners T. Cooper, F. Oslund, N. Williams, and A. Strzelczyk; and USGS partners B. Bodenstein, D. Grear, and H. Ip for their assistance in meeting our sample goals and diagnostic testing needs. We thank the MN.IT GIS experts B. Anderson and S. Benson for their GIS assistance. We thank P. Taskash, D. Schueller, and D. Rose for their help with information dissemination and media contacts. We also thank A. Fojtik and D. Stallknecht from UGA SCWDS. We thank the UMN College of Veterinary Medicine faculty L. Minicucci and veterinary residents J. Evanson and J. Lee for their assistance in field sampling. We are certain we missed some people and for that we apologize. We also thank all of the turkey hunters willing to allow us to sample their harvested animals and the citizens willing to report sick or dead birds that we screened for sampling.

LITERATURE CITED

Hildebrand, E., M. Carstensen, and E. Butler. 2010. Surveillance for highly pathogenic avian influenza in Minnesota's migratory wild birds from 2006-2010. Pages 203–211 i*n* G. DelGiudice, M. Grund, J. Lawrence, and M. Lenarz, Summaries of wildlife research findings, 2010. Minnesota Department of Natural Resources, Wildlife Populations and Research Unit, St. Paul, MN.

Ip, H.S., M.K. Torchetti, R. Crespo, P. Kohrs, P. DeBruyn, K.G. Mansfield, T. Baszler, L. Badcoe, B. Bodenstein, V. Shearn-Bochsler, M.L. Killian, J.C. Pedersen, N. Hines, T. Gidlewski, T. DeLiberto, and J.M. Sleeman. 2015. Novel Eurasian highly pathogenic avian influenza A H5 viruses in wild birds, Washington, USA, 2014. Emerging Infectious Diseases 21: 886-890.

Pasick, J., Y. Berhane, T. Joseph, V. Bowes, T. Hisanaga, K. Handel, and S. Alexanderson. 2015. Reassortant highly pathogenic Influenza A H5N2 virus containing gene segments realted to Eurasian H5N8 in British Columbia, Canada, 2014. Scientific Reports 5, 9484.

World Health Organization. 2015. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO. [cited 2015 September 5]. http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/

World Organisation for Animal Health. 2014. Summary of immediate notifications and follow-ups–2014. Highly pathogenic avian influenza [cited 2014 Dec 14]. http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary

Table 1. Description of 17 types of locations that Minnesota Department of Natural Resources staff sampled for waterfowl feces, which was subsequently submitted for avian influenza testing in spring 2015.

Description of Sampling Sites
Foam baiting stations in ditches, ponds, and marshes
Mowed grass and gravel around ponds or along dikes
Top of gravel or grass dikes
Waste water ponds
Lake sand bars
Mud flats
Vegetation mats
Golf courses
Mowed road ditches along roads
Gravel or glass shore along lakes
Upland hay meadow
Sandy lake beaches
Loafing rocks in open water and logs along shoreline
Cleared areas along lakes
Softball fields
Residence yards
Park grounds

Table 2. Species of wild bird morbidity and mortality samples (n = 104) submitted to the Minnesota Department of Natural Resources for avian influenza testing from March to August 31 2015. One Cooper's hawk tested positive for highly pathogenic avian influenza and one American coot (*Fulica americana*) tested positive for low pathogenic avian influenza.

Agency	Species sampled	*n*
MNDNR	American coot	4
	American crow	1
	Bald eagle	5
	Belted kingfisher	1
	Blackbird	2
	Broad-winged hawk	1
	Canada goose	11
	Cedar waxwing	1
	Cooper's hawk	7
	Finch	1
	Great horned owl	3
	Hawk	3
	Herring gull	1
	Mallard	3
	Mourning dove	1
	Common nighthawk	1
	Osprey	1
	Pelican	1
	Ring-necked pheasant	7
	Rail, warbler, and coot	1
	Red-tailed hawk	3
	Ring-billed gull	2
	Rock dove	1
	Rose-breasted grosbeak	1
	Sandhill crane	2
	Sharp-shinned hawk	9
	Starling	3
	Swainson's thrush	1
	Trumpeter swan	4
	Unknown passerine	1
	Wild turkey	19
	Wood duck	2
Total		104

* Note that multiple birds may have been submitted for a given location and time and samples submitted represent one batch

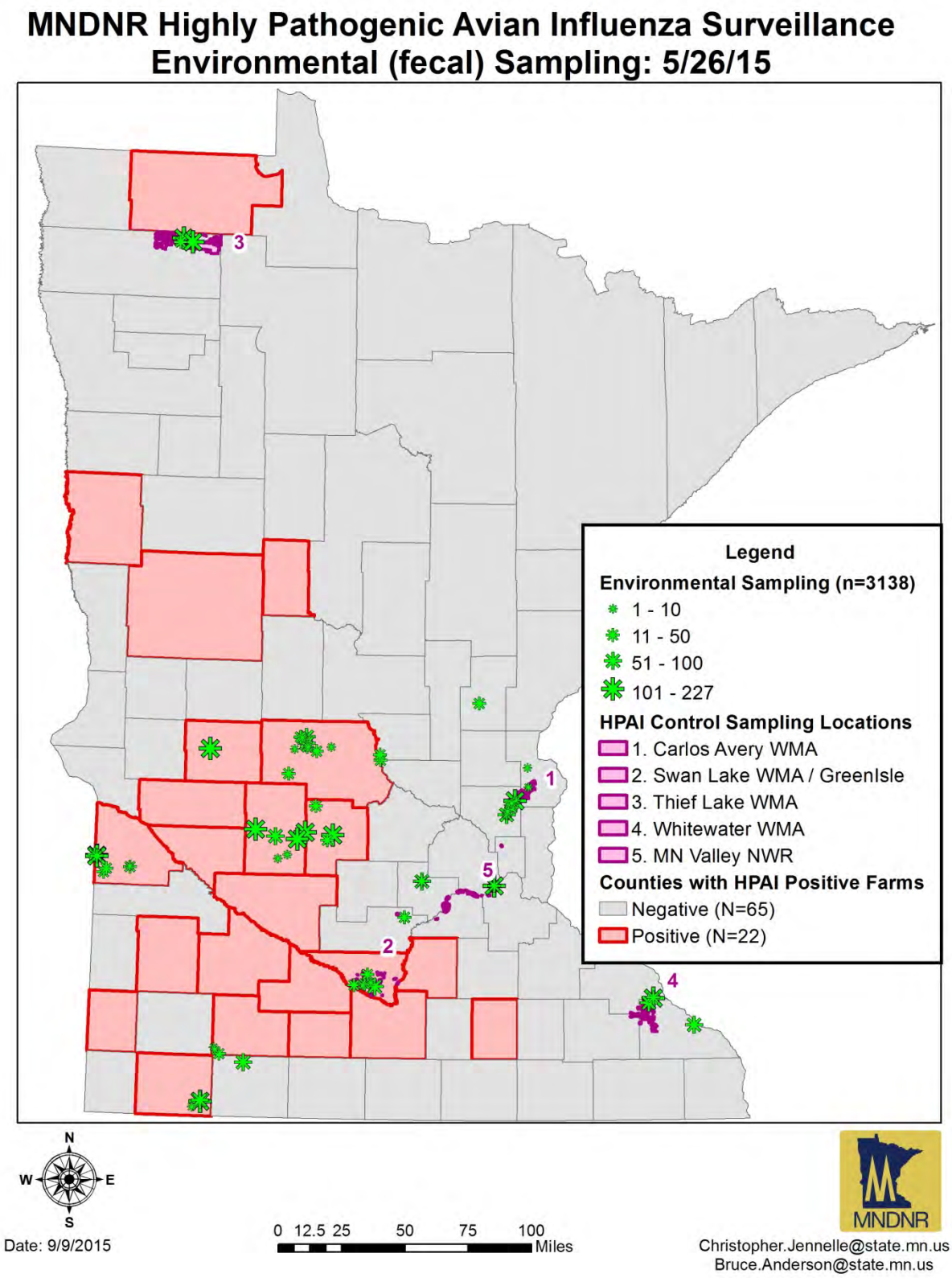

Figure 1. Sites in Minnesota where the Minnesota Department of Natural Resources collected waterfowl feces samples that were subsequently tested for highly pathogenic avian influenza by the United States Department of Agriculture in 2015.

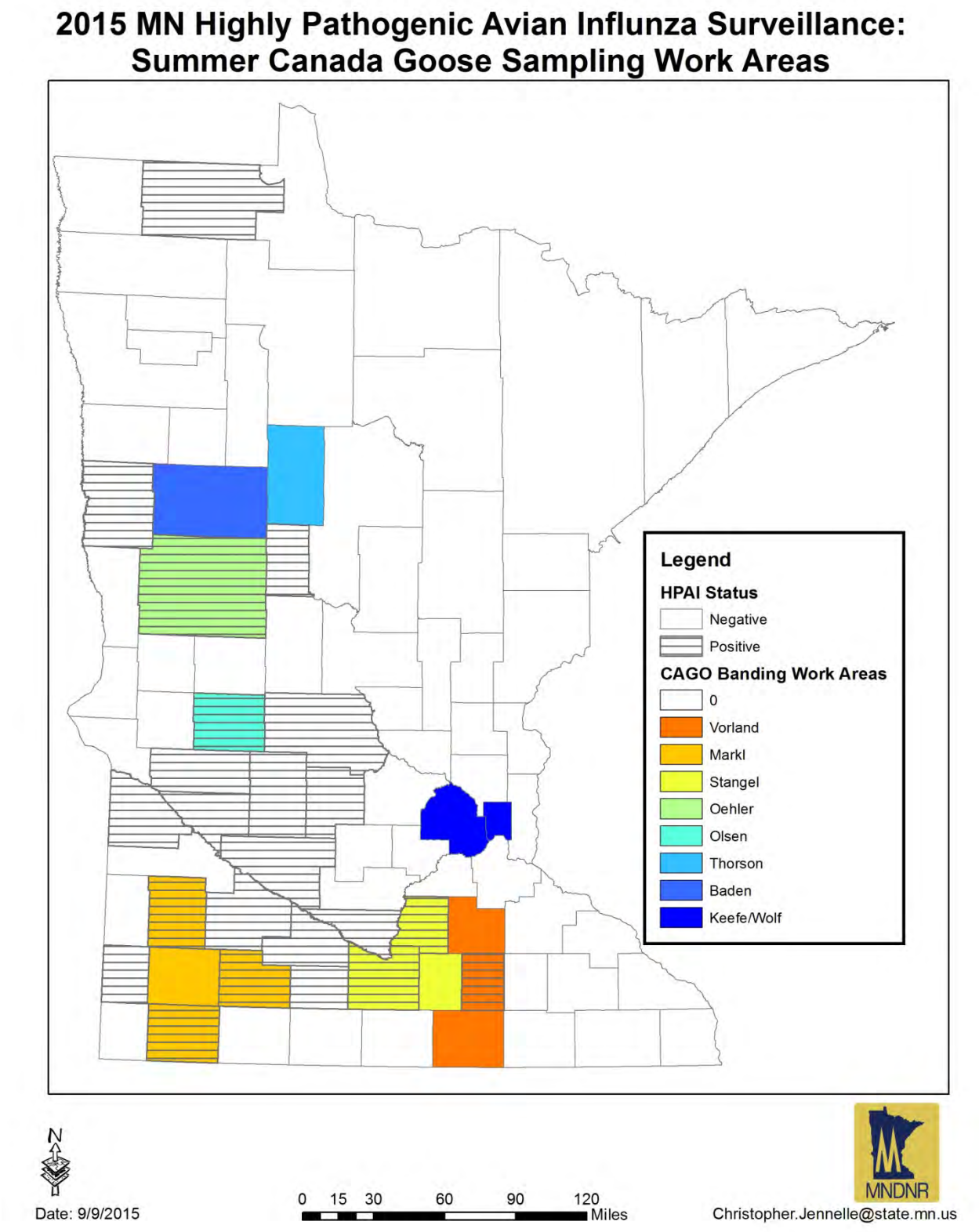

Figure 2. Study area design for summer 2015 paired collection and testing of Canada goose (*Branta Canadensis*) swab and blood samples in collaboration with SCWDS. A total of 619 goose samples were subsequently collected with 0 HPAI positive cases, 2 LPAI positive cases, and pending serology results.

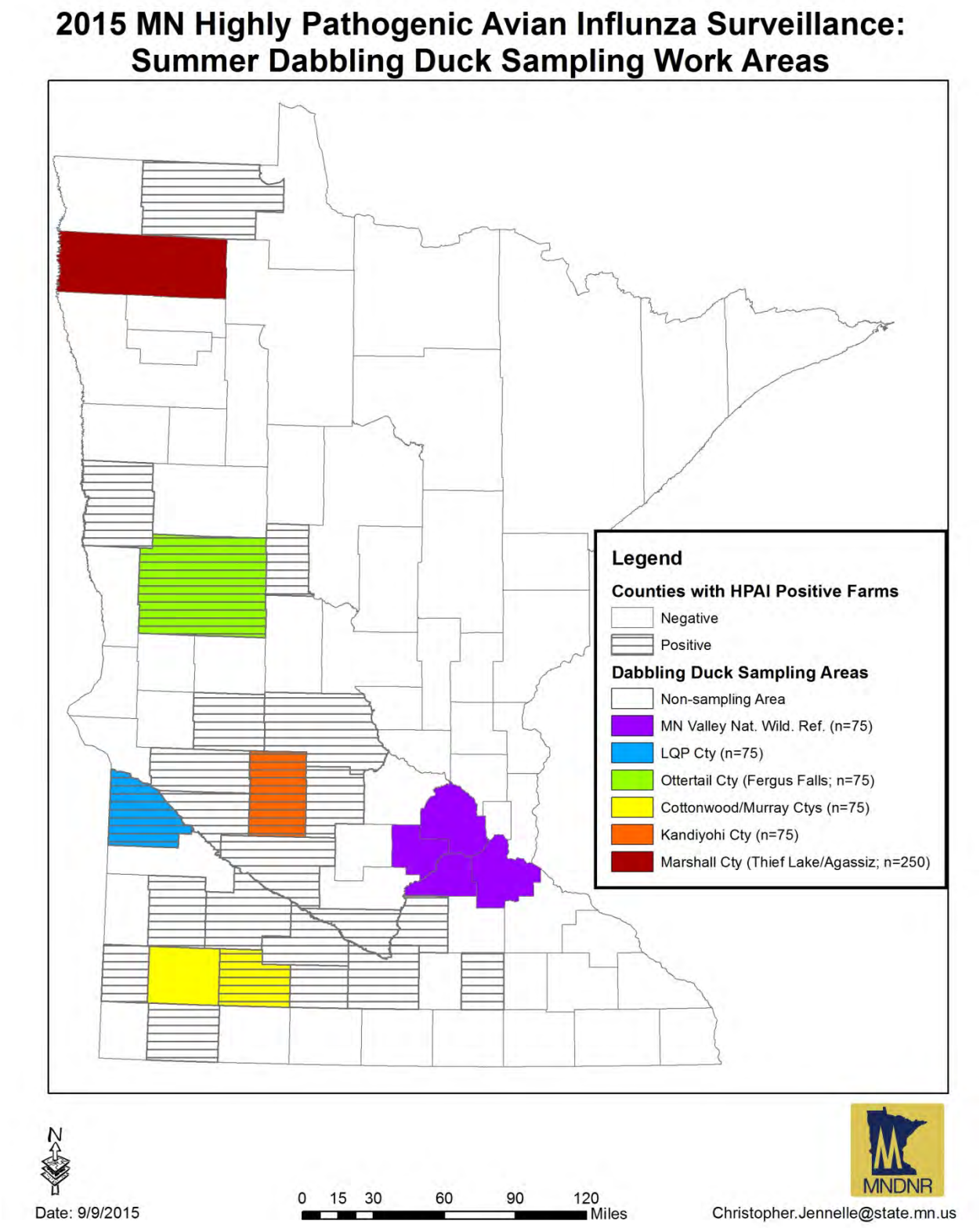

Figure 3. Study area design for summer 2015 paired collection and testing of dabbing duck swab and blood samples in collaboration with SCWDS. A goal of 625 paired samples is established in six work areas throughout Minnesota, and sampling is currently underway.

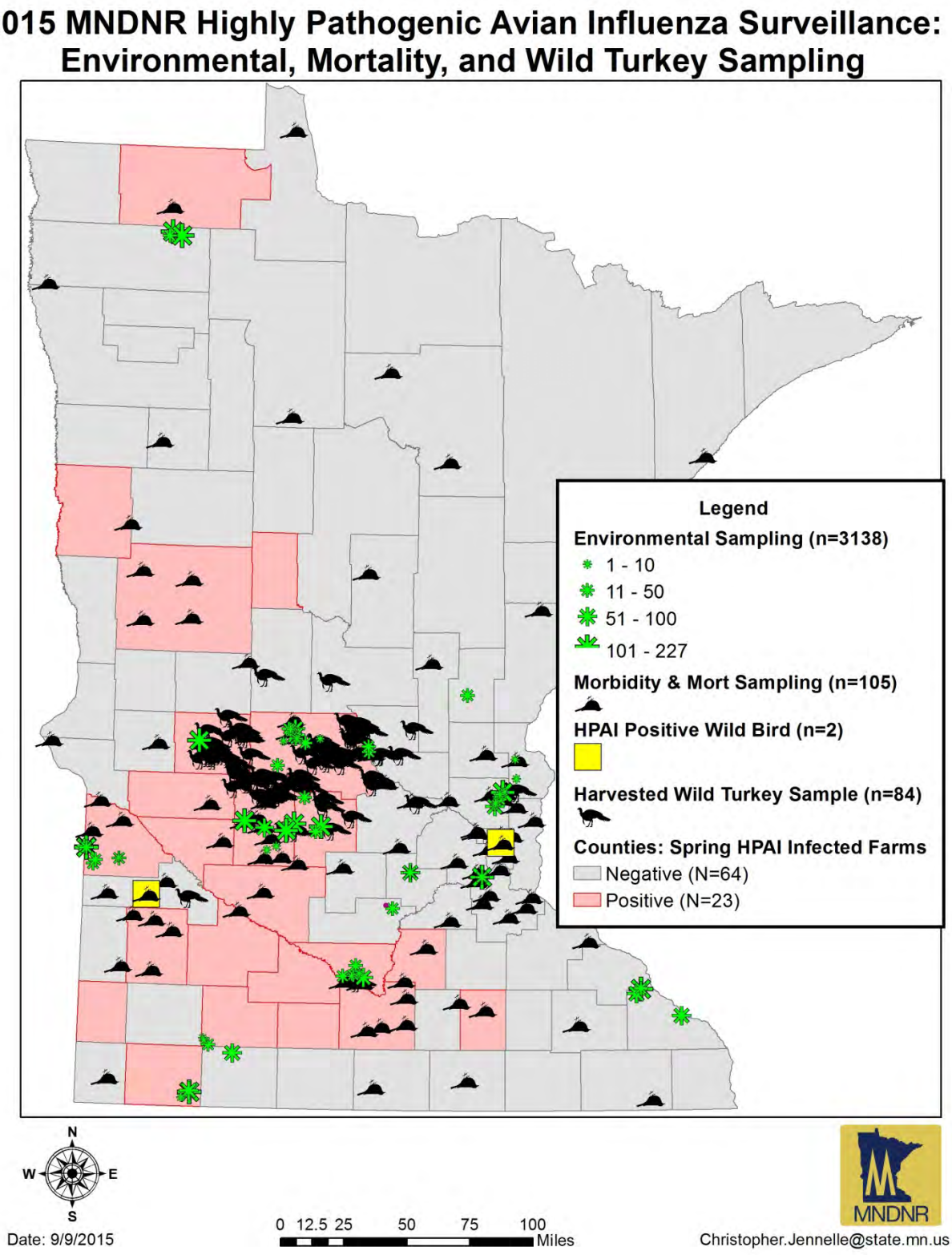

Figure 4. The distribution of waterfowl fecal, wild bird mortality, and hunter-harvested wild turkeys samples collected and tested in MN by the MNDNR through 8/31/15. Note that one of the HPAI positive wild bird mortalities (black-capped chickadee) in Ramsey County was obtained by the MN Wildlife Rehabilitation Center.

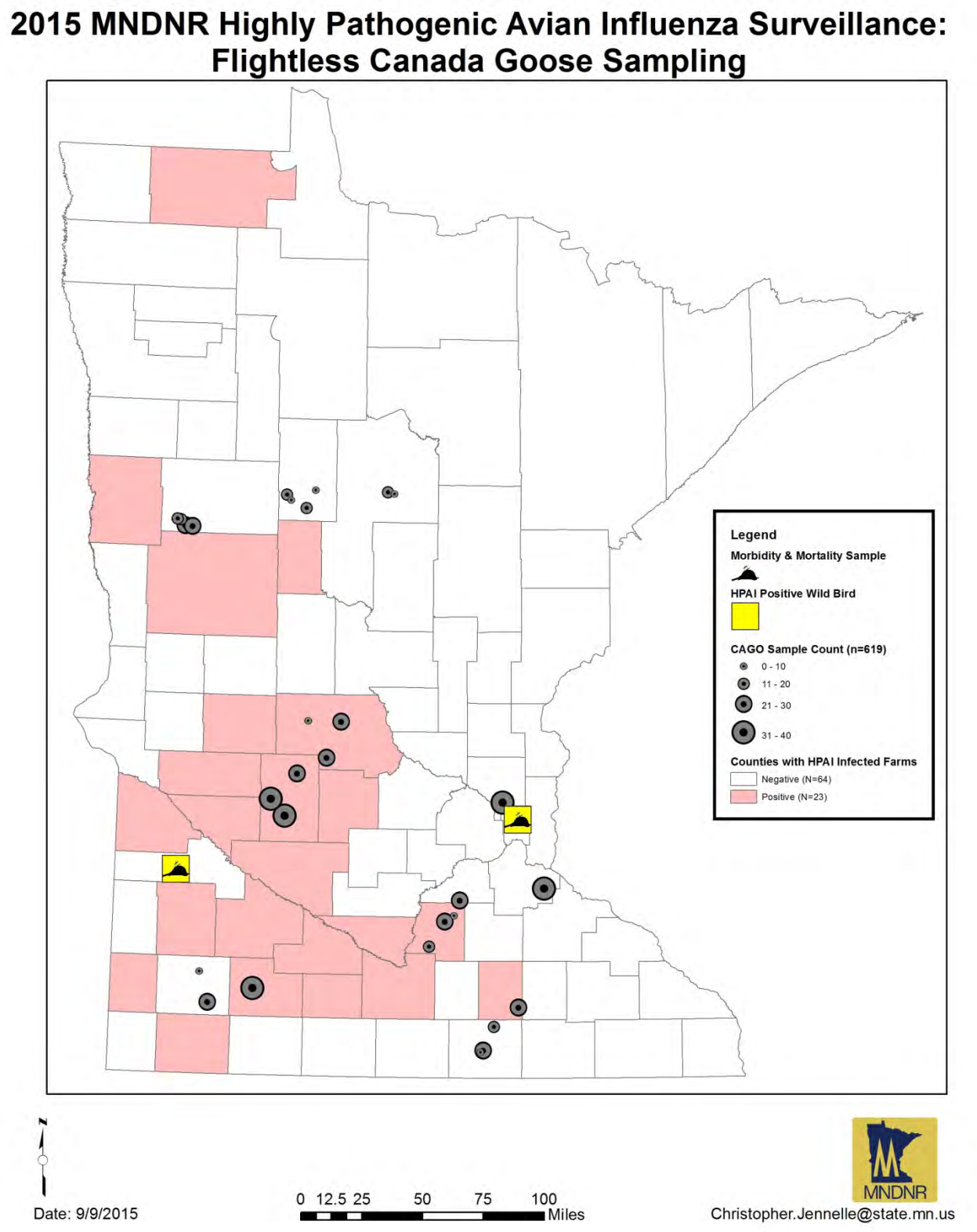

Figure 5. The distribution of 619 Canada goose paired swab and serology samples collected by the MNDNR in collaboration with SCWDS. All samples were collected between June and July 2015, and serology results are pending.

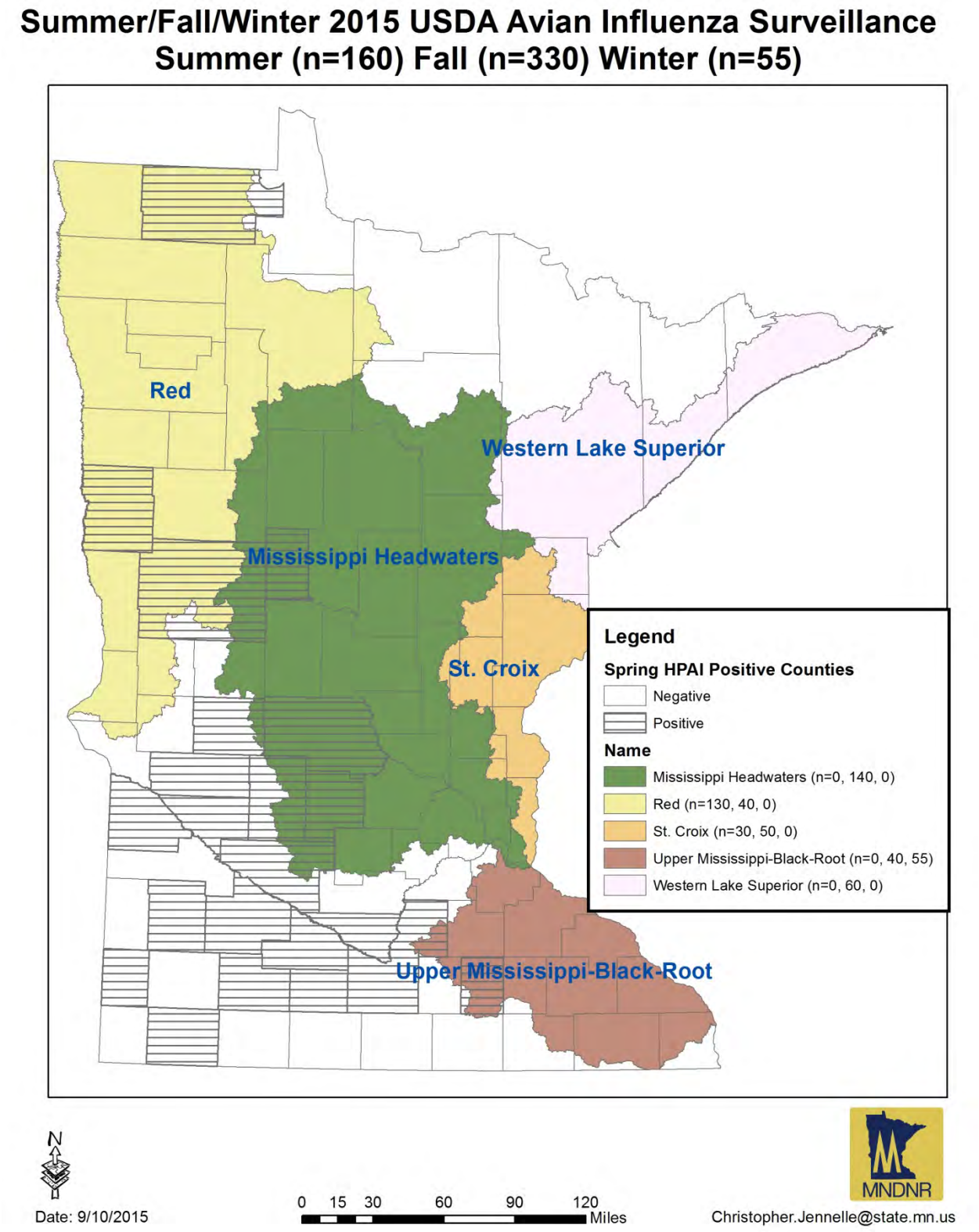

Figure 6. The distribution of USDA targeted MN watersheds for avian influenza sampling in summer, fall, and winter 2015. The three sample sizes noted beside watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively.

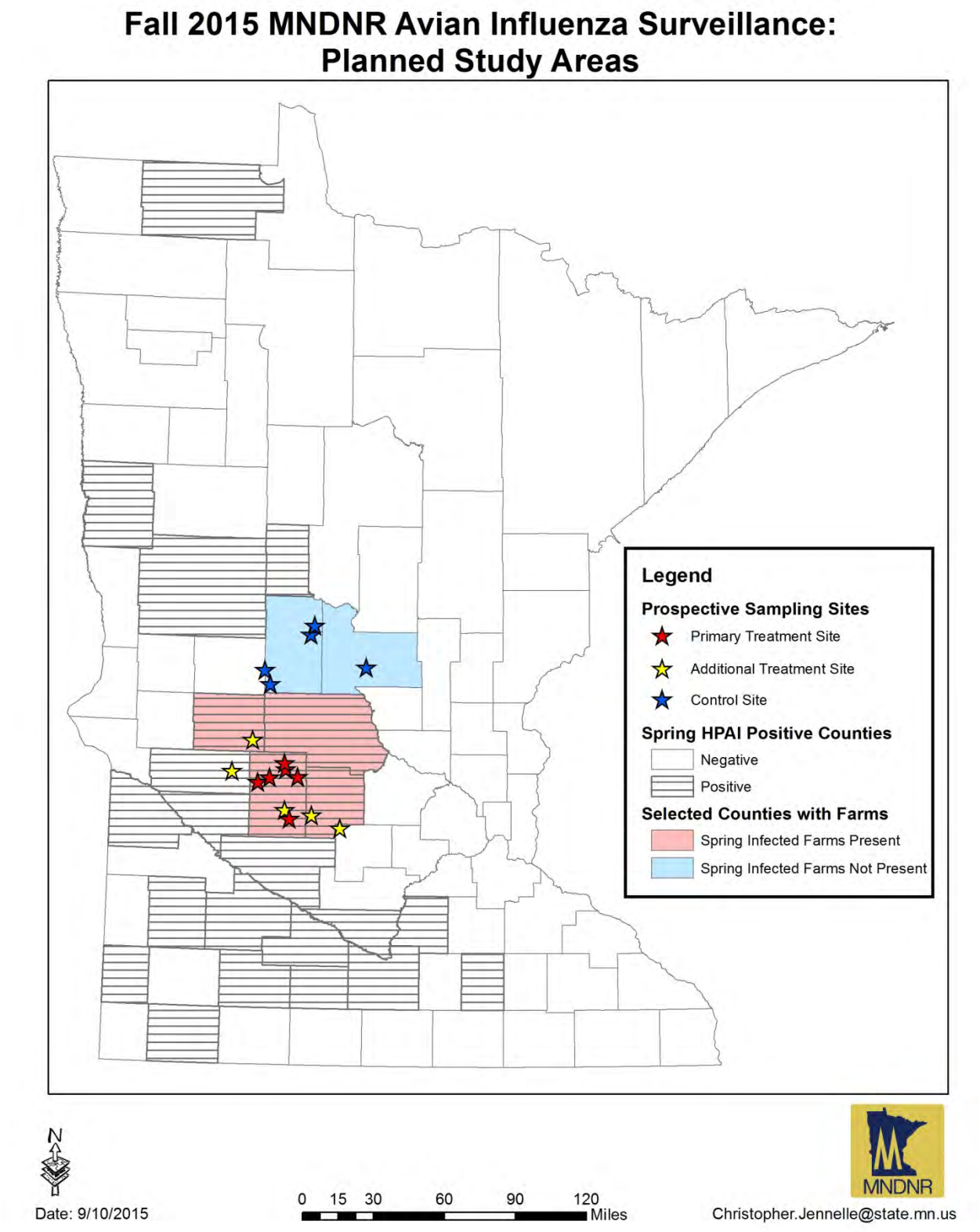

Figure 7. The distribution of MNDNR targeted counties and sampling locations for planned MN fall duck hunter-harvest sampling for avian influenza. Approximately 400 tracheal and cloacal swab samples will be collected per bird in each of the two study area types.

CHRONIC WASTING DISEASE SURVEILLANCE IN MINNESOTA'S SOUTHEASTERN WILD DEER HERD

Erik Hildebrand[1], Michelle Carstensen, Margaret Dexter, and Chris Jennelle

SUMMARY OF FINDINGS

In fall 2014, the Minnesota Department of Natural Resources (MNDNR) sampled 411 hunter-harvested white-tailed deer (*Odocoileus virginianus*) for chronic wasting disease (CWD) in southeastern Minnesota. The surveillance effort focused on testing deer within deer permit areas (DPA) 348 and 349, in response to the first detection of CWD in a free-ranging deer by the Iowa Department of Natural Resources in Allamakee County. All deer were negative for the disease. MNDNR also submitted samples from 69 deer from within DPA's 236 and part of 601 (north metro surveillance area) where a captive European red deer *(Cervus elaphus)* farm was found positive for CWD in summer 2012. These deer were collected through vehicle kills, special hunts, and depredation permits; all deer were negative for CWD. In addition, MNDNR submitted samples from 18 cervids through targeted surveillance, which included sick animals, escaped captive cervids, and vehicle-kills; these were also all negative for CWD. Currently, MNDNR has suspended efforts to test for CWD through hunter-harvested surveillance in the state, but will continue with targeted surveillance efforts.

INTRODUCTION

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) that affects elk *(Cervus elaphus)*, mule deer *(Odocoileus hemionus)*, white-tailed deer, and moose *(Alces alces)*. TSEs are infectious diseases that alter the morphology of the central nervous system, resulting in a "sponge-like" appearance of this tissue. The etiological agent of CWD is an infectious protein, called a prion. Incubation time of the disease can range from 1.5 to nearly 3 years, although infected animals have been shown to shed prions in their feces up to a year before showing signs of illness (Tamguney et al. 2009, Haley et al. 2011). Clinical signs are non-specific and may include a loss of body condition and weight, excessive salivation, ataxia, and behavioral changes. There is no known treatment or vaccine for the disease and it is always fatal. Experimental and circumstantial evidence suggest that transmission of the disease is primarily through direct contact with infected animals or their infective saliva or excrement (Mathiason et al. 2006, Safar et al. 2008). However, persistence of prions in the environment and resulting indirect transmission has been shown to occur (Miller et al. 2004, Johnson et al. 2007, and Maluquer de Motes et al. 2008).

The Center for Disease Control (CDC) and other public health agencies have concluded there is no known link between CWD and any neurological disease in humans (MaWhinney et al. 2006, Sandberg et al. 2010). However, both the CDC and the World Health Organization (WHO) recommend that no part of a known positive animal should be consumed by humans. Additionally, there is no evidence that CWD can be naturally transmitted to species other than deer, elk, or moose.

To date, CWD has been diagnosed in 3 captive elk *(Cervus canadensis)* herds, 1 captive white-tailed deer herd, and 1 captive European red deer (*Cervus elaphus)* herd in Minnesota. Two of the elk herds (Stearns and Aitkin counties) were discovered in 2002 and depopulated; no additional CWD-positive animals were found. In 2006, a captive white-tailed deer from a mixed deer/elk herd in Lac Qui Parle County was infected with CWD and depopulated without additional infection being detected. In 2009, another captive elk herd (Olmsted County) was found infected

[1] Corresponding author email: erik.hildebrand@state.mn.us

with CWD and, following depopulation of >600 animals, a total of 4 elk were confirmed with the disease. The United States Department of Agriculture's (USDA) indemnification document noted there was an apparent longstanding infection within this captive elk facility. In 2012, a captive European red deer was found infected with CWD in a herd of approximately 400 animals in North Oaks, MN. This marked the first time CWD was discovered in this species. This red deer herd was depopulated in 2014; no additional infected animals were found. According to the indemnity agreement, perimeter fences must remain intact at this property until 2019, in an effort to keep wild deer from entering the property to reduce disease transmission risks.

Currently, Minnesota has approximately 500 captive cervid facilities. As the current statewide population estimate of wild deer approaches one million, there is an element of inherent disease transmission risk between captive and wild cervids. Overall, risk is difficult to quantify because deer populations are unevenly distributed over the landscape ranging in densities from < 1-15 deer/km^2 (i.e., 1–40 deer/mi^2), facility fences vary in construction quality, and direct/indirect contact rates between captive and wild cervids are unknown. In addition, captive cervid facilities are sporadically distributed on the landscape and are independent of wild deer densities.

In November 2010, MNDNR sampled 564 hunter-harvested deer focused on a 32.2-km (20-mi) radius around a CWD-positive captive elk facility near Pine Island (Olmstead county), discovered in 2009. One free-ranging deer tested positive for CWD, marking the first detection of the disease in Minnesota's wild deer population. In response to this disease detection, MNDNR conducted a fixed-wing aerial deer survey in a 16.0-km (10-mi) radius of the index case in late January 2011 and estimated 6,200 deer (7.3 deer/km^2 or 19 deer/mi^2). A supplemental surveillance effort was conducted in February–March 2011; 752 adult deer were sampled and all tested negative. To prevent further disease spread, MNDNR banned recreational feeding of deer in a 4-county area in southeastern Minnesota and created a CWD Management Zone DPA 602. From 2011–2013, a total of 4,050 (n = 1,125, 1,195, and 978 for 2011, 2012, and 2013, respectively) deer were sampled for CWD within DPA 602 with no further infection detected.

These data, in combination with historical data from 2002-2009 indicated >99% probability that disease prevalence was no greater than 0.5% assuming independence between years and animals within year. These results provide strong evidence that Minnesota was on the front end of a CWD outbreak in wild deer. Our inability to detect any additional infected deer in the immediate vicinity of the index case or in surrounding DPA's or in DPA's bordering neighboring infected counties is encouraging. The data suggests CWD was recently introduced on the southeastern MN landscape, with a high likelihood that widespread wild cervid exposure has been minimal.

METHODS

Hunter-harvested surveillance during 2014 was conducted at deer registration stations during the first two weekends of the regular firearm hunting season in southeastern Minnesota. Selected stations were staffed with MNDNR personnel and students (veterinary medicine and natural resources) trained in lymph node collection. Stations were selected based on deer volume and distribution throughout the surveillance zone to meet a sampling goal of 450 between DPAs 348 and 349 combined. Hunters were asked to voluntarily submit medial retropharyngeal lymph node samples from deer ≥1.5 years of age to be tested for CWD, and a front incisor was extracted from all deer visually assessed to be ≥2.5 years old for aging by cementum annuli. To obtain access to deer from the north metro surveillance area MNDNR worked with local contractors and the Wildlife Science Center to collect vehicle-killed deer within a 10-mile radius of the CWD-infected red deer farm in North Oaks. Additional deer were obtained through special hunts in Ramsey and Anoka counties, as well as both private and city depredation permits. All deer samples were inventoried, entered into a database, and sent to Colorado State University (Fort Collins, CO) for enzyme-linked immunosorbent assay (ELISA) testing. Any presumptive positive deer from ELISA testing would be confirmed using immunohistochemistry (IHC) testing at the National Veterinary Services Laboratory in Ames, Iowa.

At the time when deer were sampled, hunter information was recorded, including the hunter's name, a telephone number, MNDNR number, and location of harvest. Maps were provided to assist the hunters in identifying the location (Township, Range, and Section) of the harvest site. Cooperating hunters were given a cooperator's patch.

Across MN, MNDNR consistently samples any cervid exhibiting clinical symptoms of CWD

infection (targeted surveillance). We have disseminated information to wildlife staff regarding clinical signs of infection for symptomatic deer. We also provided staff with the necessary equipment and training for lymph node removal and data recording. The number of samples expected through targeted statewide surveillance is estimated to be less than 100 animals annually, as few reports of deer with clinical signs are received.

RESULTS AND DISCUSSION

MNDNR collected a total of 411 samples in southeastern Minnesota from hunter-harvested deer during fall 2014 (Figure 1). All samples were negative for CWD. The sampling goal was 450 samples between DPA's 348 and 349 combined, and we achieved 91% of our surveillance goal in southeastern MN.

From July 2014 to June 2015, MNDNR collected a total of 18 samples from targeted surveillance efforts. This included samples from 2 escaped captive deer, and 16 free-ranging deer with clinical signs; all samples were negative for CWD.

In the north metro surveillance area, 69 deer were tested in fall 2014. From 2012 – 2014, a total of 350 (160, 121, and 69, respectively) deer were tested for CWD through vehicle-kills (*n*=48), special hunts (*n*=163), and from a city-contracted sharpshooting effort within the city of North Oaks (*n*=139), with no detection of the disease (Figure 2).

Hunter-harvested deer was and remains the primary source for obtaining adequate samples for continued monitoring and management of this disease since the first discovery of CWD in MN in 2002. MNDNR remains concerned about CWD spread in wild cervids, and has increased surveillance focus in southeastern Minnesota with evidence of increasing CWD detections in wild deer in southwestern Wisconsin and northeastern Iowa.

Future Surveillance Plans

Given there have been no CWD detections in hunter-harvested wild deer since 2010, and no detections via targeted surveillance efforts, MNDNR will not conduct hunter-harvest surveillance in 2015. Targeted CWD surveillance of deer exhibiting clinical signs of illness will continue statewide.

ACKNOWLEDGMENTS

We would like to thank all the MNDNR Wildlife and Enforcement staff, who volunteered to assist with this disease surveillance project. We also wish to thank the students and faculty from the University of Minnesota, Colleges of Veterinary Medicine and Natural Resources, for assisting in our fall sampling efforts. Special thanks to Julie Hines and Bob Wright for fulfilling our GIS mapping needs. We appreciate the support of the USDA-Wildlife Services disease biologist Paul Wolf.

LITERATURE CITED

Haley, N., C. K. Mathiason, S. Carver, M. Zabel, G. C. Telling, and E. A. Hoover. 2011. Detection of Chronic Wasting Disease Prions in Salivary, Urinary, and Intestinal Tissues of Deer: Potential Mechanisms of Prion Shedding and Transmission. Journal of Virology 85(13): 6309-6318.

Johnson, C. J., J. A. Pederson, R. J. Chappell, D. McKenzie, and J. M. Aiken. 2007. Oral transmissibility of prion disease is enhanced by binding to soil particles. PLoS Pathogens 3:e93.

Maluquer de Motes, C., M. J. Cano, M. Pumarola, and R. Girones. 2008. Detection and survival of prion agents in aquatic environments. Water Research 42:2465-2472.

Mathiason, C. K., J. G. Powers, S. J. Dahmes, D. A. Osborn, K. V. Miller, R. J. Warren, G. L. Mason, S. A. Hays, J. Hayes-Klug, D. M. Seelig, M. A. Wild, L. L. Wolfe, T. R. Spraker, M. W. Miller, C. J. Sigurdson, G. C. Telling, and E. A. Hoover. 2006. Infectious prion in the saliva and blood of deer with chronic wasting disease. Science 314:133-136.

MaWhinney, S., W. J. Pape, J. E. Forster, C. A. Anderson, P. Bosque, and M. W. Miller. 2006. Human prion disease and relative risk associated with chronic wasting disease.

Emerging Infectious Diseases 12:1527-1535.

Miller, M.W., E. S. Williams, N. T. Hobbs, and L. L. Wolfe. 2004. Environmental sources of prion transmission in mule deer. Emerging Infectious Diseases 10:1003–1006.

Tamguney, G., M. W. Miller, L. L. Wolfe, T. M. Sirochmann, D. V. Glidden, C. Palmer, A. Lemus, S. J. DeArmond, and S. B. Prusiner. 2009. Asymptomatic deer excrete infectious prions in feces. Nature 461:529–532.

Safar, J. G., P. Lessard, G. Tamguney, and Y. Freyman. 2008. Transmission and detection of prions in feces. Journal of Infectious Diseases 198:81-89.

Sandberg, M. K., H. Al-Doujaily, C. J. Sigurdson, M. Glatzel, C. O'Malley, C. Powell, E. A. Asante, J. M. Linehan, S. Brandner, J. D. F. Wadsworth1 and J. Collinge. 2010. Chronic wasting disease prions are not transmissible to transgenic mice overexpressing human prion protein. Journal of General Virology 91: 2651–2657.

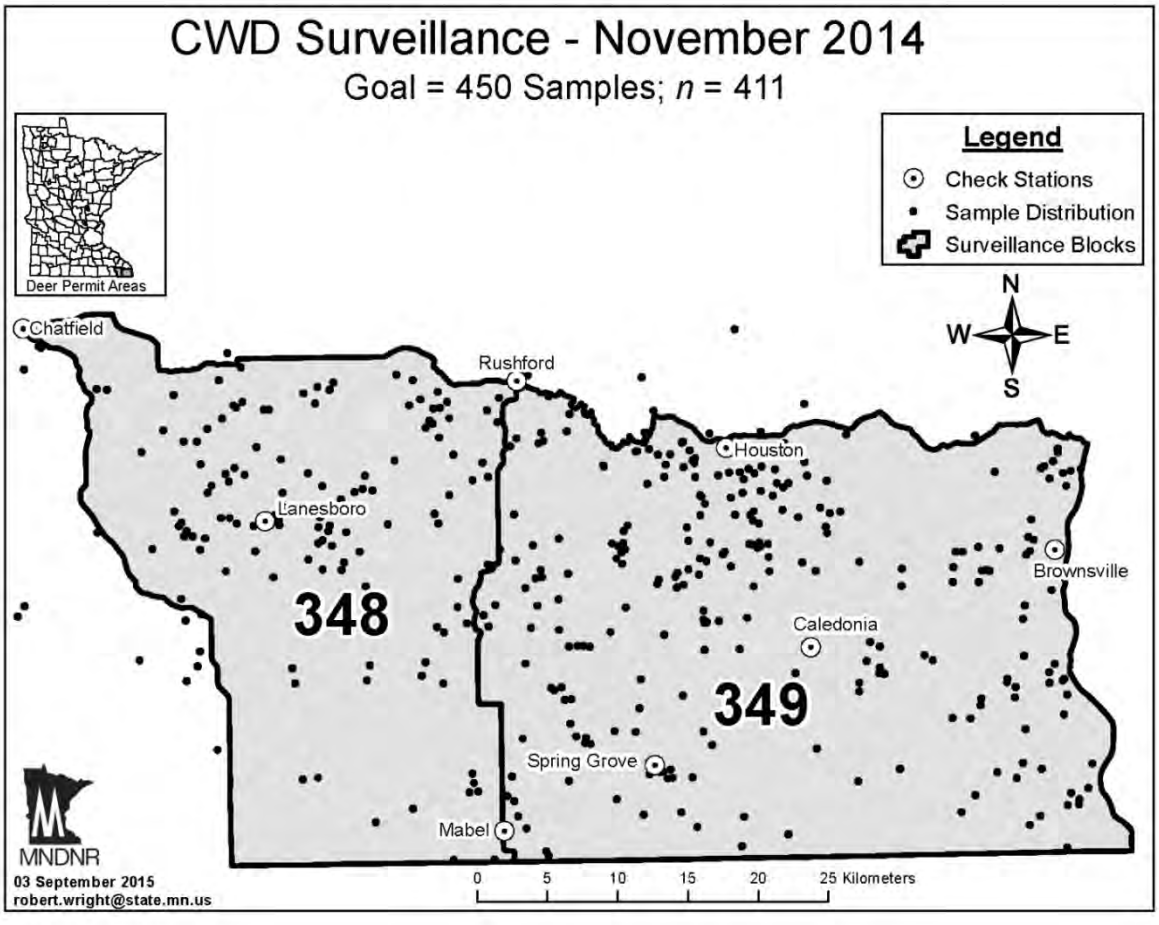

Figure 1. Samples collected from deer *(n*=411*)* for chronic wasting disease (CWD) testing in southeastern Minnesota during fall 2014.

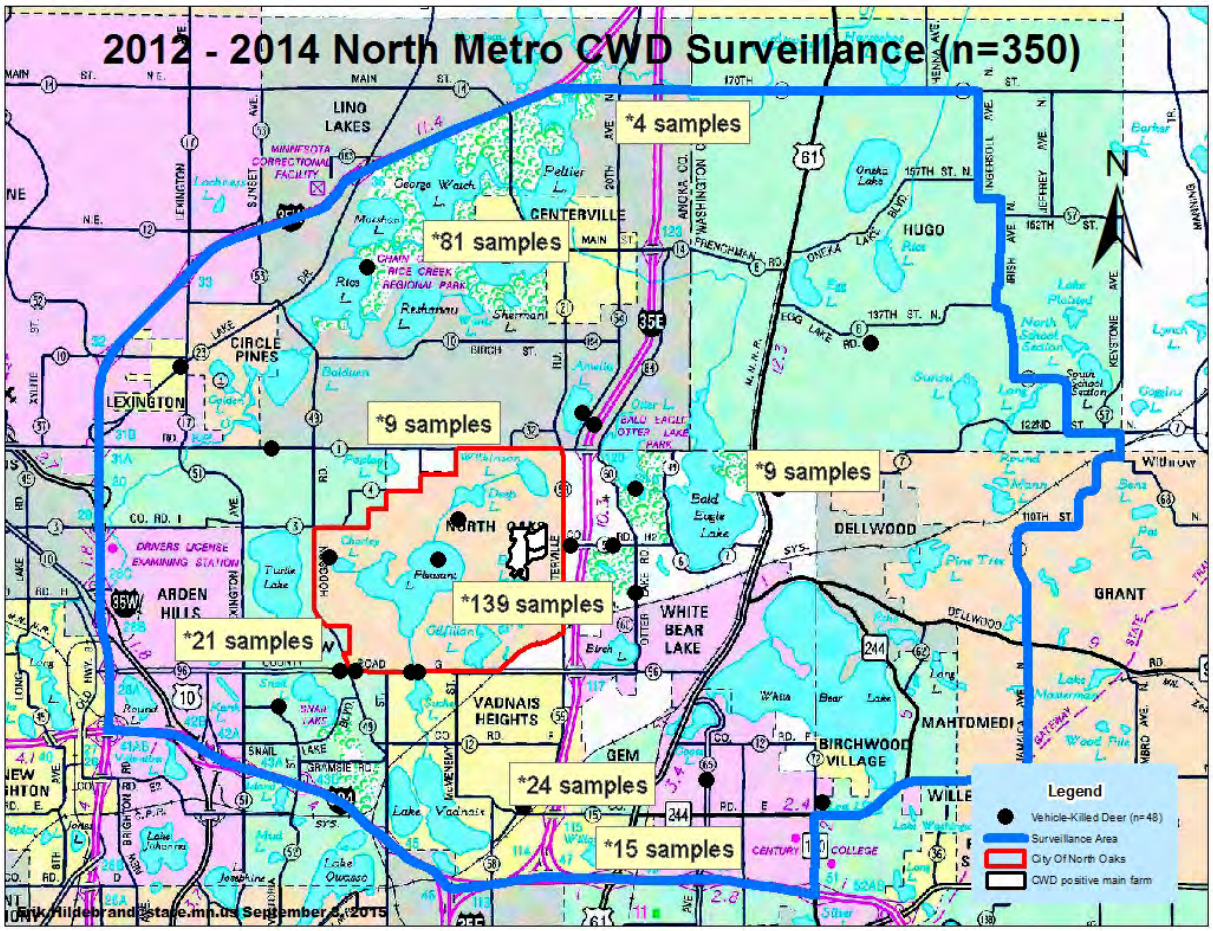

Figure 2. Samples collected from deer *(n*=350*)* for chronic wasting disease (CWD) testing in the north metro surveillance area, in relation to the location of CWD-positive European red deer farm, 2012 through fall 2014.

MNDNR

DETERMINING CAUSE- SPECIFIC MORTALITY OF ADULT MOOSE IN NORTHEAST MINNESOTA

Michelle Carstensen, Erik C. Hildebrand, Dawn Plattner, Margaret Dexter, Christopher Jennelle, and Robert G. Wright (Minnesota IT Services)

SUMMARY OF FINDINGS

The primary goal of this study is to improve our understanding of the causes of non-hunting mortality in northeastern Minnesota's declining moose (*Alces alces*) population. Our goal is to respond to potential mortalities within 24 hours of death, prior to decomposition of tissues. In the first 2.5 years of this multiyear study, we've captured and radio collared 173 adult moose (123 females, 50 males), and mean age at capture (n = 120, some ages still pending) was 5.7 years (±0.3 yrs; range = 1 to 14). A total of 41 collared moose have died since this study began, excluding 12 capture-related mortalities that will be censored from subsequent survival analyses. Annual mortality varied from 19% in 2013 to 12% in 2014; the 2015 mortality rate to date is 9%. Overall proximate causes of death included: 16 confirmed and likely wolf kills (39%), 8 bacterial infections (20%), 5 confirmed and likely *Parelaphostrongylus tenuis* infections (12%), 4 multiple, chronic health issues (10%), 3 winter tick infestations (7%), 1 accident (2%), and 4 undetermined health issues (10%). Whole carcasses were retrieved for 13 (32%) of mortalities, with field necropsies performed on the remaining 28 (68%) moose. Response times from initial mortality notification (e.g., text message or email) to a team in the field at the death site were ≤24 hours in 25 cases (61%), between 24 and 48 hours in 11 cases (27%), and >48 hours in 5 cases (12%). Delays in mortality responses > 24 hours have been due to collar failures and wolves actively feeding on the moose carcass and preventing the collar from sending a mortality alert. There are currently 115 remaining moose in the study, but 30 of these have collars that are experiencing significant transmission failures and we are not certain of their status; thus, 85 moose are actively transmitting data.

INTRODUCTION

Historically, moose were found throughout the forested zone of north central Minnesota. By the 1960's there were two distinct populations, the northwest (NW) population of the aspen parklands and northeast (NE) population of the boreal forest (Fuller 1986). In the mid-1980's the NW population began a precipitous decline, falling from 4,000 to <100 animals by the early 2000's (Murray et al. 2006, Lenarz 2007). Murray et al. (2006) identified pathogens, including liver flukes (*Fascioloides magna*) and brainworm (*P. tenius*), as the principal cause of death for 37-62% of radio-collared animals; 25% of additional mortalities were likely pathogen-induced, but limited necropsy evidence was inconclusive. They also observed that many moose in NW MN dying of natural causes were malnourished, as evidenced by 51% of carcasses having bone marrow fat (BMF) contents below a critical threshold (< 30%) and trace mineral deficiencies (i.e., copper and selenium).

Subsequently, in NE MN, Lenarz et al. (2009) reported a 21% average non-hunting mortality rate for radiocollared moose, which was much higher than the 8-12% reported for moose elsewhere in North America (Larsen et al. 1989, Ballard 1991, Kufeld and Bowden 1996). Specific causes of most of the non-anthropogenic mortality (89%) could not be determined, as assessing cause-specific mortality was not the primary objective of the study

(Lenarz et al. 2009). Many of the deaths appeared health-related, with prime age animals dying during unusual times of the year or carcasses found intact with little evidence of scavenging.

Aerial surveys also indicate the NE population is declining. Since the estimated peak at 8,840 moose in 2006, the 2015 estimated moose population (3,450) is 61% lower and time series analysis of estimates since 2006 indicate a significant downward trend (DelGiudice 2015). Butler et al. (2013) documented evidence of exposure of NE MN moose to a variety of disease agents (e.g., West Nile Virus, eastern equine encephalitis, malignant catarrhal fever), which could be potential mortality factors. Additionally, a recent study of sick and vehicle-killed moose (*n*=62) from 2003-2013 had found 85% of animals were undernourished and infected with a variety of disease agents, including brainworm (45%), liver flukes (60%), and winter ticks (*Dermacentor albipictus*) (22%) (Wuenschmann et al., 2014). Researchers have hypothesized that brainworm was responsible for historic declines in moose populations (Karns 1967, Prescott 1974, Lankester 1987), but it is questionable whether brainworm currently represents a major threat to the NE MN population; clinical signs consistent with brainworm infection were first reported in MN moose in 1912 (Fenstermacher and Olson 1942). Lenarz et al. (unpublished data) found that brainworm may have caused an average 19% (0-32%) of the population's total annual mortality. Recently, Mech and Fieberg (2014) have suggested that wolves (*Canis lupus*) had a stronger role in the northeast moose decline than previously reported.

Climate change has been implicated as an underlying factor in both population declines. There were inverse relationships between warming ambient temperatures and decreasing survival of adult moose or negative rates of population change (Murray et al. 2006; Lenarz et al. 2009, 2010). Trends in temperature and precipitation patterns are likely to increase in intensity over the next century (Houghton et al. 2001). If moose are unable to sufficiently thermoregulate above certain ambient temperature thresholds (Renecker and Hudson 1986, 1990; McCann et al., 2013) we might expect to see increased body temperatures and energy expenditures required to stay cool, which over time could have negative consequences for body condition, reproduction, and survival. Currently, no data exist to support the direct adverse effects of ambient temperature on the physiology, survival, or reproduction of free-ranging moose. Recently, a minimally invasive telemetry system for ruminants, called a mortality implant transmitter (MIT), has been developed to allow nearly continuous monitoring of body temperature with a battery lifetime of ≥2 years. Using these MITs and global positioning system (GPS) collars on adult moose in this study will allow us to correlate ambient temperature with physiology, behavior (habitat use and activity), and fitness (survival and reproduction). This study will be the first to examine these relationships in a way that includes monitoring body temperature. The results of this study will be critical to determine if moose modify their activity and use available habitat in response to ambient temperatures, and to evaluate population performance.

METHODS

Moose were captured within the study area (Figure 1) by aerial darting (Quicksilver Air Inc., Alaska) with carfentanil (4.0, 4.5mg or 6.0mg), or thiafentanil (16mg) and xylazine (150mg or 30mg) from a helicopter; immobilizations were reversed with naltrexone (425-575mg) and tolazoline (400mg). Blood (serum and whole blood) was collected at capture by venipuncture of the jugular vein. Serum was screened for evidence of exposure to 10 disease agents following the same protocol as described by Carstensen et al. (2014). Additionally, serum was submitted for a large animal serum chemistry profile and reproductive hormones to assess physiological status, overall health, and pregnancy status (Franzmann and LeResche 1978, Haig et al. 1982, Duncan et al. 1994). Serum progesterone levels were determined by the Smithsonian Institute; levels >2.0 ng/mL were considered pregnant. Whole blood in Ethylenediamine tetraacetic acid (EDTA) was used to make blood smears and complete differential blood cell counts were performed, which may be indicative of condition and health status (Duncan et al. 1994), presence of tick-borne illnesses, and evaluation for the presence of microfilaria. An incisor (I4) was removed for aging by cementum annuli (Sergeant and Pimlott 1959). A general fecal

floatation examination for parasites was performed. A thorough physical examination was performed, including assessment of body condition score (very thin, thin, normal, fat), winter tick load, and hair loss. Rump fat measurements (Maxfat, cm) were measured by portable ultrasound to further assess body condition and nutritional status (Cook et al. 2010, DelGiudice et al. 2011). Total body length and girth (cm) were measured to estimate body weight of moose (Hundertmark and Schwartz, 1998) and hair samples were collected from the withers. Any mortalities that occurred within two weeks of capture were censored from the study.

Moose were fitted with mortality-sensing collars utilizing GPS and Iridium two-way communication technologies (Vectronic Aerospace GmbH; Berlin, Germany). Collars transmit location and status data to a base station (Forest Lake, MN) at user-defined intervals. The base station also analyzes location data to identify animals that have "localized" (e.g., remained within a 20m radius for >24 hours), to assist with detecting sick animals that are potentially moribund. When a mortality or localization event occurred, the mortality response team was notified via text and email messages. Mortality implant transmitters (Vectronic Aerospace GmbH) were placed orally into a subset of the captured moose. These devices are similar to a cow magnet in size, log internal temperatures every 15 minutes, and transmit a subset of this data through the collar. Additionally, MITs are meant to provide immediate notification of mortality via detection of minimal internal activity (e.g., lack of a heart beat) and this notification is also sent via text and email message to the moose mortality response team. External temperature loggers (Hobo TibdbitV2; Onset Corporation, Bourne, MA) were affixed to the GPS collar and were programmed to collect ambient temperature every 60 minutes.

Moose mortality response teams have 8 primary team leaders that have undergone extensive necropsy training, and they are supported by about 20 secondary and tertiary team members (including MNDNR, tribal, academic, US Forest Service, and other personnel) available upon request. Every effort is made to remove carcasses intact from the field and deliver them to the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL) for a complete necropsy by a board-certified pathologist. Teams are able to utilize special equipment, including trucks with 2000lb winches, an amphibious ARGO, chainsaw winch (e.g. Lewis winch), heavy duty snow machines with long tracks, all-terrain vehicles, and specialized rubber mats with built-in hitches for dragging carcasses. Primary members have also taken specialized training with DNR forestry and fire units to be able to sling out a moose carcass via helicopter. If a moose was found to be alive, but obviously ill, it was euthanized (via gunshot). If carcass extraction was not possible, a thorough and complete field necropsy was performed, guided by an established protocol. Samples were submitted to the UMN VDL for diagnostic evaluation (Carstensen et al. 2014).

RESULTS AND DISCUSSION

Annual survival and timing of mortalities

A total of 41 collared moose (33 females, 8 males) have died since this study began; which excludes 12 capture-related mortalities that are censored from subsequent survival analyses. Overall proximate causes of death were as follows: 16 confirmed and likely wolf kills (39%), 8 bacterial infections (20%), 5 confirmed and likely *P. tenuis* infections (12%), 4 multiple, chronic health issues (10%), 3 winter tick infestations (7%), 1 accident (2%), and 4 undetermined health issues (10%; Figure 2). A third of the wolf-killed moose had significant health conditions that likely predisposed them to predation, including encephalitis and meningitis in the brain*, P. tenuis* infections, and pneumonia in the lungs. Health-related causes were attributed to 61% of total deaths, with the remaining 39% being predator-related. Timing of these mortalities suggest that most deaths occur in spring (54%, March–May); however, moose died in all seasons (Winter 17%, Summer 22%, and Fall 7%; Figure 3). Annual (January–December) survival rate was 81% and 89% in 2013 and 2014, respectively; 91% of moose have survived from January–August 2015 (Figure 4). There are currently 115 remaining moose in the study, but 30 of these have collars that are experiencing significant transmission failures and we are not certain of their status; thus, 85 moose are actively transmitting data.

A total of 12 collared moose were censored due to capture-related mortalities, 4 (3.6%), 3 (8.1%), and 5 (15.6%) in 2013, 2014, and 2015, respectively. Unfortunately, the elevated number of captured-related deaths experienced in 2015 resulted in our decision to discontinue captures and not deploy 9 additional collars for Dr. Ron Moen's study as we had intended. Capture myopathy was the primary cause of these deaths; however, the exact mechanism involved that led to these mortalities has not been identified. Immobilizing drugs and dosages were consistent with previous years' captures in MN and comparable to other moose capture efforts in North America and Scandinavia. Pursuit and handling times averaged 6 and 55 minutes, respectively, which was similar to capture efforts in 2013 with 12% less capture-related mortalities.

Overall age of moose (*n*=35) at death was 7.8 years (±0.7 year), with ages still pending from the remaining 6 moose that died during this study. Mean age of moose that died from health-related causes (*n*=17) was 8.1 years (±1.0 year), similar to those (*n*=18) that died of wolf-related causes (7.5 ±1.1 years). Interestingly, both health and predator-related causes of death impacted nearly every age cohort in this study (Figure 5), which suggests that wolves are not selecting for just young (<3 years of age) or old (>8 years of age) moose and are able to prey upon prime aged (4-8 years old) individuals as well.

Thus far in 2015, the moose mortality rate is similar to 2014 and only half of what occurred in 2013. It appears that winter survival was enhanced by the prolonged winter 2013, which may have suppressed winter tick numbers, as we have not reported any winter tick mortalities during winter 2014 or 2015. Also, the 2 consecutive, historically severe, winters of 2014 and 2015 likely reduced deer numbers in our study area, which would lessen disease exposure risks of moose to *P. tenuis* and liver flukes.

Health Screening at Capture

Pregnancy rate was 89% in 2015; higher than 2013 (83%) and 2014 (77%). Moose at capture in 2015 (*n*=32) were generally in good body condition (44% normal, 53% thin, and 3% very thin). Ultrasonic rump fat measurements were obtained from 25 moose, and maximum rump fat averaged 1.05cm (SE = 0.17cm). There was minimal hair loss noted from winter ticks. Overall, nutritional condition in moose at capture was the poorest in 2013, but average to good in 2014 and 2015, as evidenced by the combination of body condition scores, ultrasonic rump fat measurements, and snow urine chemistries.

Overall exposure to West Nile Virus (29/158, 18%), Eastern Equine Encephalitis (0/158, 0%), various serovars of *Leptospira interrogans(*23/158, 15%), malignant catarrhal fever (51/158, 32%), and *Borreilia* (27/158, 17%) were reported. While blood evidence indicated exposure to these various diseases (with the exception of EEE), clinical evidence of infection was not observed either during capture or at death in applicable cases. Little is still known about how these diseases may impact moose or contribute to reduced survival or productivity. Further analyses of serum chemistries are pending.

Mortality Response Times

Whole carcasses were retrieved for 13 (32%) of the study cohort, with field necropsies preformed on the remaining 28 (68%) moose. Response times from initial mortality notification (e.g. text message or email) to a team in the field at the death site were ≤24 hours in 25 cases (61%), between 24 and 48 hours in 11 cases (27%), and >48 hours in 5 cases (12%). Delays in mortality responses > 24 hours have been due to collar failures and wolves actively feeding on the moose carcass and preventing the collar from sending a mortality alert.

Mortality Implant Transmitters

We successfully deployed 61 MITs in moose during this study. An additional 20 MITs were spit out by moose shortly after oral application. This rate of MIT rejection was markedly reduced from 40% (12 of 20) in 2014 to 13% (3 of 23) in 2015 due to improved application methods that included a specialized bolus applicator and properly timed reversal of xylazine to ensure the swallowing reflex was intact.

In December 2014, we began a MIT calibration project with the Moose Research Center within Alaska's Department of Game and Fish. Thus far, the study has shown the MIT to be a highly accurate measurement of internal body temperature in moose. On average, the MIT was only 0.25°C higher than body temperature determined by vaginal implant transmitters. Further, preliminary analyses of data from MITs recovered from moose that have died in Minnesota (*n*=8) indicated prolonged elevated temperatures (>102°F) for 10-30% of readings during the summer months.

Management Implications

This aggressive study has demonstrated that it is possible to respond to moose mortalities in a timely manner and obtain valuable diagnostic information to help illuminate the many causes of death for this species. The use of satellite-GPS technology was instrumental in allowing us to identify mortality events; however, we had to overcome some significant challenges in collar functionality. Previous studies in MN have pointed to health impacts as a potential driver in population declines; yet, many of those deaths lacked diagnostic data to assign causation. In this study, we not only obtained diagnostic evidence of parasites and pathogens, we also identified predisposing conditions that may be contributing to proximate causes of mortality (e.g., a brainworm infection moose is then killed by wolves). Another example is the documentation of 4 cases of initial wolf-induced injuries that did not result in immediate death, rather the moose lived for several days to weeks before succumbing to bacterial infection from that initial attack. It is likely that this type of mortality has been largely undiscovered or underestimated in previous studies, given the often lengthy time delays in getting to carcasses to obtain needed evidence. Further, 61% of all proximate mortalities in this study were health-related and 39% were predator-related. Teasing apart some of the ultimate causes (e.g. toxicities, pathogens, parasites, and climate change) will require more data over a longer time-span, as we are only 2.5 years into this current study.

Funding and Future Project Direction

This 2 million dollar project initially began through funding from the Legislative Citizen Commission on Minnesota's Resources (LCCMR; $600,000) and in-kind contributions from the MNDNR, University of Minnesota, tribal partners, and nonprofit organizations (e.g. MDHA, Northstar Museum). This project was also funded in part by the Wildlife Restoration Program (Pittman-Robertson). Additional funding through LCCMR (ENRTF project "Moose Decline and Air Temperatures in Northeastern Minnesota", M.L. 2014, Chp. 226, Sec. 2, Subd. 5m) was recently secured for $600,000 to continue the study and expand on the use of MITs to gain insight into the potential role ambient temperature may play in moose survival and productivity. Sample size of at least 100 collared adult moose has been maintained over 3 years (2013-2015) and no additional capture operations are planned at this time. Collars deployed in 2015 should last 4-5 years, meaning this study could continue until 2020

ACKNOWLEDGMENTS

This project is very demanding and would not be possible without the assistance of the following groups and individuals: the Environment and Natural Resources Trust Fund for funding the majority of this project, Dr. Arno Wuenshmann and Dr. Anibal Armien (UM VDL) for their diagnostic investigations of the mortalities, Mike Schrage (Fond du Lac Natural Resources) and Andy Edwards (1854 Treaty Authority) for their assistance in the field and during captures, Richard Gerhold and Caroline Grunenwald (University of Tennessee) for assisting with the identification of microfilaria and *P. tenuis*, Ulrike Munderloh (University of MN, Department of Entomology) for testing samples for tick-borne illness, J. P. Dubey (USDA, ARS) for neospora and toxoplasma testing, our team of primary responders (Dave Pauly, Nancy Hansen, Dave Ingebrigtsen, and John Giudice; MNDNR), our team of secondary responders (Bob Fashingbauer, Bob Kirsch, Bryan Lueth, Carolin Humpal, Jim LaBarre, Leslie McInenly, Lindsey Shartell, Meadow Kouffeld-Hansen, Steve Piepgras, Tim Pharis, Tom Rusch, Ted Dick, Penny Backman, Jessica VanDuyn, Bailey Petersen, Marshall Deters, and Jeff Hines; MNDNR), Dan Ryan and Dave Grosshuesch (US Forest Service), Brandon Seitz (Grand Portage National

Monument), EJ Issac and Seth Moore (Grand Portage Band), Lance Overland (Fond du Lac Resources), Nick Bogyo (1854 Treaty Authority), Bill Severud and Tyler Obermoller (Univ of MN) for their assistance in the field, and the MNDNR enforcement pilots (Jason Jensen, John Heineman, Tom Buker, and Bob Geving) for their assistance during captures, USDA-Wildlife Services (Paul Wolf) for use of their necropsy trailer, and Tyler Obermoller, Kaytee Firnett, Jeanna Lodel, Beth Martin, Amanda McGraw, and Amy Kingsley for assistance with data management and gearing-up for captures. Rob Fasteland (MNDNR Forestry) and the Lake & Cook County Highway Department staff for snow plowing and maintaining helispots used during capture events. Special thanks to special operations staff for remote hook/sling and radio training, including Bill Schuster, Lee Kessler, Mike McLaughlin, Dustin Nelson and Pat Coughlin.

LITERATURE CITED

Ballard, W.B., J.S. Whitman, and D.J. Reed. 1991. Population dynamics in south-central Alaska. Wildlife Monograph 114.

Butler, E.A., M. Carstensen, E. Hildebrand, and J. Giudice. 2013. Northeast Minnesota moose herd health assessment 2007–2012. Minnesota Department of Natural Resources [MNDNR]. http://www.dnr.state.mn.us/publications/wildlife/research2012.html

Carstensen, M., E. C. Hildebrand, D. C. Pauly, R. G. Wright, and M. H. Dexter. 2014.. Determining cause-specific mortaltiy in Minnesota's northeast moose populations. Pages 133–143 i*n* L. Cornicelli, M. Carstensen, M. Grund, M. Larsen, and J. Lawrence. Summaries of wildlife research findings, 2013. Minnesota Department of Natural Resources, Wildlife Populations and Research Unit, St. Paul, MN.

Cook, R.C., J.G. Cook, T.R. Stephenson, W.L. Myers, S.M. Mccorquodale, D.J. Vales, L.L. Irwin, P. Briggs Hall, R.D. Spencer, S.L. Murphie, K. A. Schoenecker, and P.J. Miller. 2010. Revisions of rump fat and body scoring indices for deer, elk, and moose. Journal of Wildlife Management 74:880-896.

DelGiudice, G.D. 2015 Aerial Moose Survey Final Results. Minnesota Department of Natural Resources [MNDNR]. 2015 DNR moose survey

DelGiudice, G. D., B. A. Sampson, M. S. Lenarz, M. W. Schrage, and A. J. Edwards. 2011. Winter body condition of moose (*Alces alces*) in a declining population in northeastern Minnesota. Journal of Wildlife Disease 47:30-40.

Duncan, J.R., K.W. Trasse, and E.A. Mahaffey. 1994. Veterinary laboratory medicine 3rd edition. Iowa State Univeristy, Ames, USA.

Fenstermacher, R. and O.W. Olson. 1942. Furthers studies of diseases affecting moose III. Cornell Veterinarian 32:241-254.

Franzmann, A.W. and R.E. LeResche. 1978. Alaskan moose blood studies with emphasis on condition evaluation. Journal of Wildlife Management 42:334-351.

Fuller, T.K. 1986. Observations of moose, Alces alces, in peripheral range in northcentral Minnesota. Canadian Field Naturalist 100:359-362.

Haigh, J.C., E. H. Kowal, W. Runge, and G. Wobeser. 1982. Pregnancy diagnosis as a management tool for moose. Alces 18:45-53.

Houghton, J. T., Y. Ding, D. J. Griggs, N. Noguer, P. J. van der Linden, X. Dai, K. Maskell, and C. A. Johnson. 2001. Contribution of working group 1 to the third assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom.

Hundertmark, K. J and C. C. Schwartz. 1998. Predicting body mass of Alaskan Moose (*Alces alces gigas*) using body measurements and condition assessment. Alces 34(1): 83-89.

Karns, P.D. 1967. Pneumostrongylus tenuis in deer in Minnesota and implications for moose. Journal of Wildlife Management 32:299-303.

Kufeld, R.C., and D.C. Bowden. 1996. Survival rates of Shiras moose (*Alces alces shirasi*) in Colorado. Alces 32:9-13.

Lankester, M.W. 1987. Pests, parasites and diseases of moose (*Alces alces*) in North America. Swedish Wildlife Research Supplement 1:461-489.

Larsen, D.G., D.A. Gauthier, and R.L. Markel. 1989. Cause and rate of moose mortality in

the southwest Yukon. Journal of Wildlife Management 53:457-481.
Lenarz, M. S., M. W. Schrage, A. J. Edwards, and M. E. Nelson. 2007. Moose population dynamics in northeastern Minnesota. Pages 346-348 *in* M. W. DonCarlos, R. O. Kimmel, J. S. Lawrence, and M. S. Lenarz, eds. Summaries of wildlife research findings, 2005. Minnesota Department of Natural Resources, Wildlife Populations and Research Unit, St. Paul.
Lenarz, M.S., M.E. Nelson, M.W. Schrage, A.J. Edwards. 2009. Temperature mediated moose survival in northeastern Minnesota. Journal of Wildlife Management 73:503-510.
McCann, N. P., R. A. Moen, and T. R. Harris. 2013. Warm-season heat stress in moose (*Alces alces*). Canadian Journal of Zoology 91: 893-898.
Mech, L. D., and J. Fieberg. 2014. Re-evaluating the northeastern Minnesota moose decline and the role of wolves. Journal of Wildlife Management 78(7): 1143-1150.
Murray, D.J., E.W. Cox, W.B. Ballard, H.A. Whitlaw, M.S. Lenarz, T.W. Custer, T. Barnett, and T.K. Fuller. 2006. Pathogens, nutritional deficiency, and climate influences on a declining moose population. Wildlife Monographs 116:1-30.
Prescott, W. H. 1974. Interrelationships of moose and deer of the genus Odocoileus. Naturaliste Canadien 101:493-504.
Renecker, L. A., and R. J. Hudson. 1986. Seasonal energy expenditure and thermoregulatory response of moose. Canadian Journal of Zoology 64:322-327.
Renecker, L. A., and R. J. Hudson. 1990. Behavioral and thermoregulatory responses of moose to high ambient temperatures and insect harassment in aspen dominated forests. Alces 26:66-72.
Sergeant, D.W., and D.H. Pimlott. 1959. Age determinationin moose from sectioned incisor teeth. Journal of Wildlife Management 23:315-321.
Wünschmann, A., A. G. Armien, E. Butler, M. Schrage, B. Stromberg, J. B. Bender, A. M. Firshman, and M. Carstensen. 2015. Necropsy findings in 62 opportunistically collected free-ranging moose (*Alces alces*) from Minnesota, USA (2003-2013). Journal of Wildlife Diseases 51(1): 157-165.

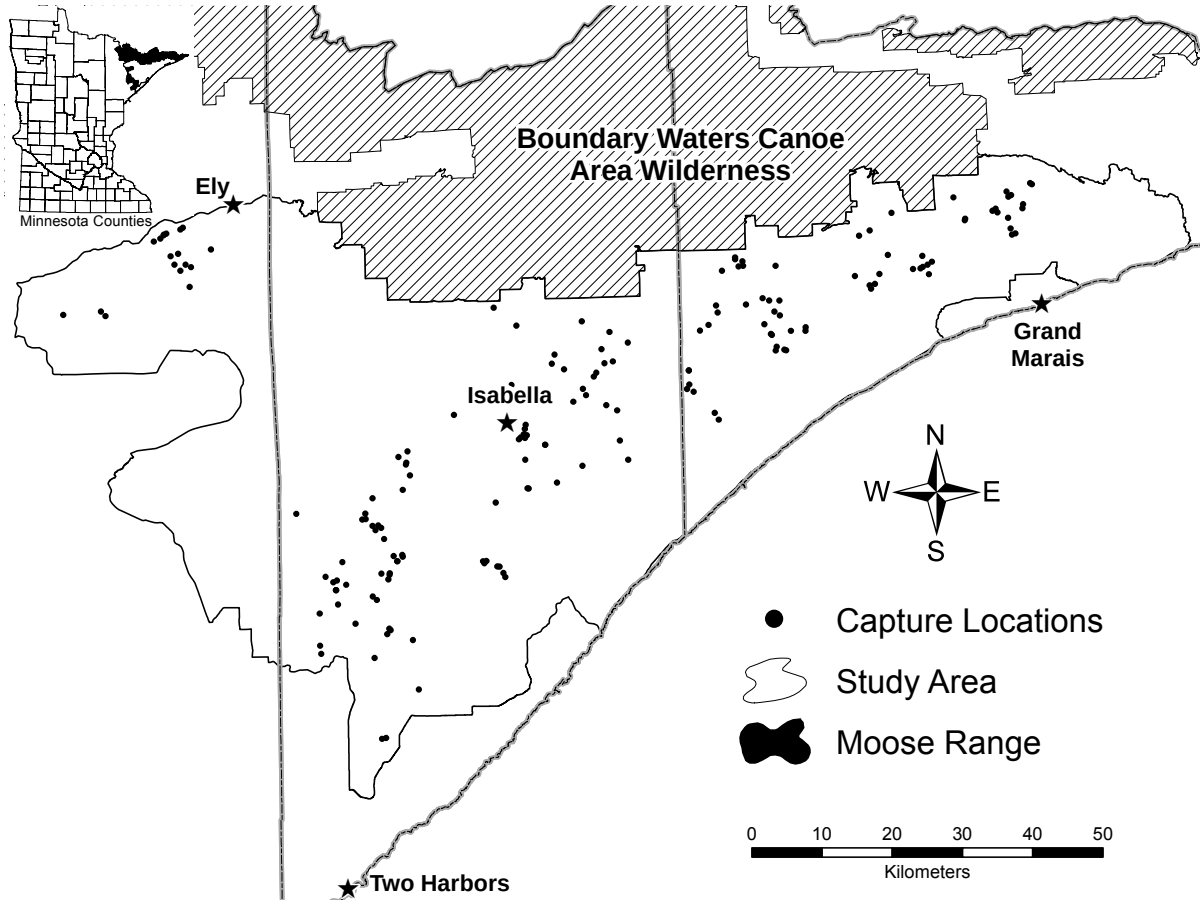

Figure 1. Study area in northeast Minnesota where 179 moose (included 6 recaptures) have been captured and radiocollared (2013–2015) to study cause-specific mortality.

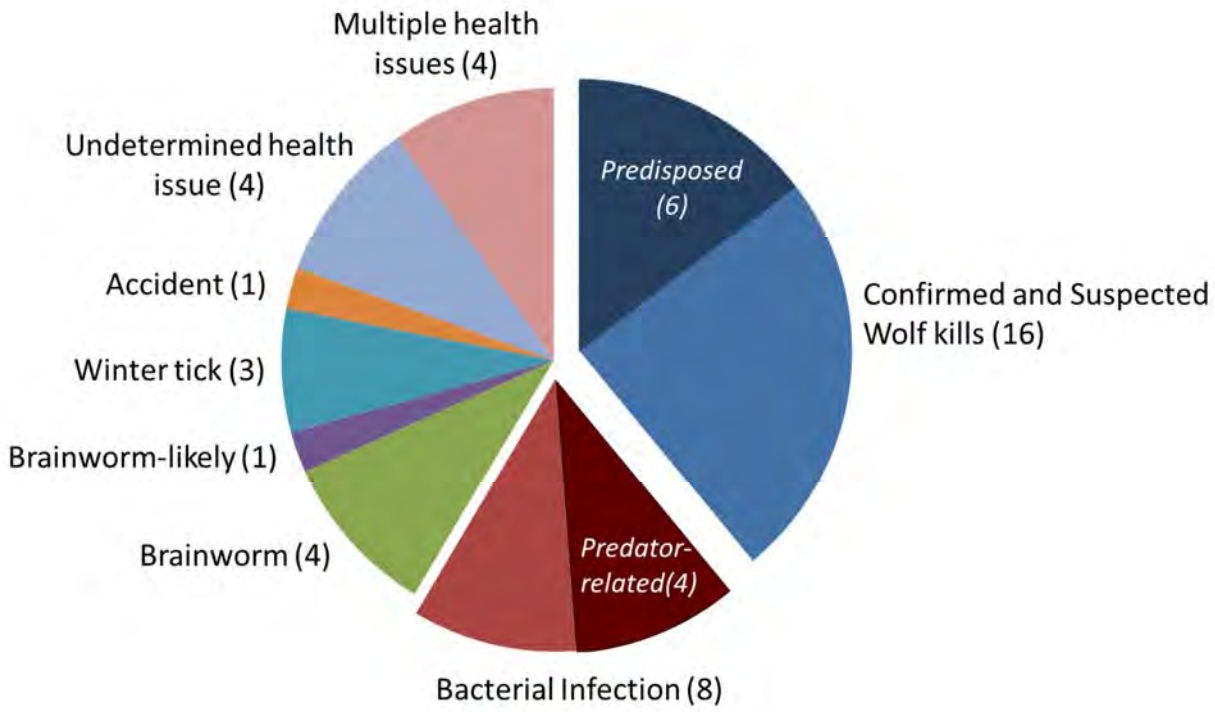

Figure 2. Cause-specific mortality of radiocollared, adult moose (*n*=41) from February 2013 to August 2015, northeast Minnesota. Predisposed wolf killed moose indicated that a significant health issue was identified that may have contributed to its death.

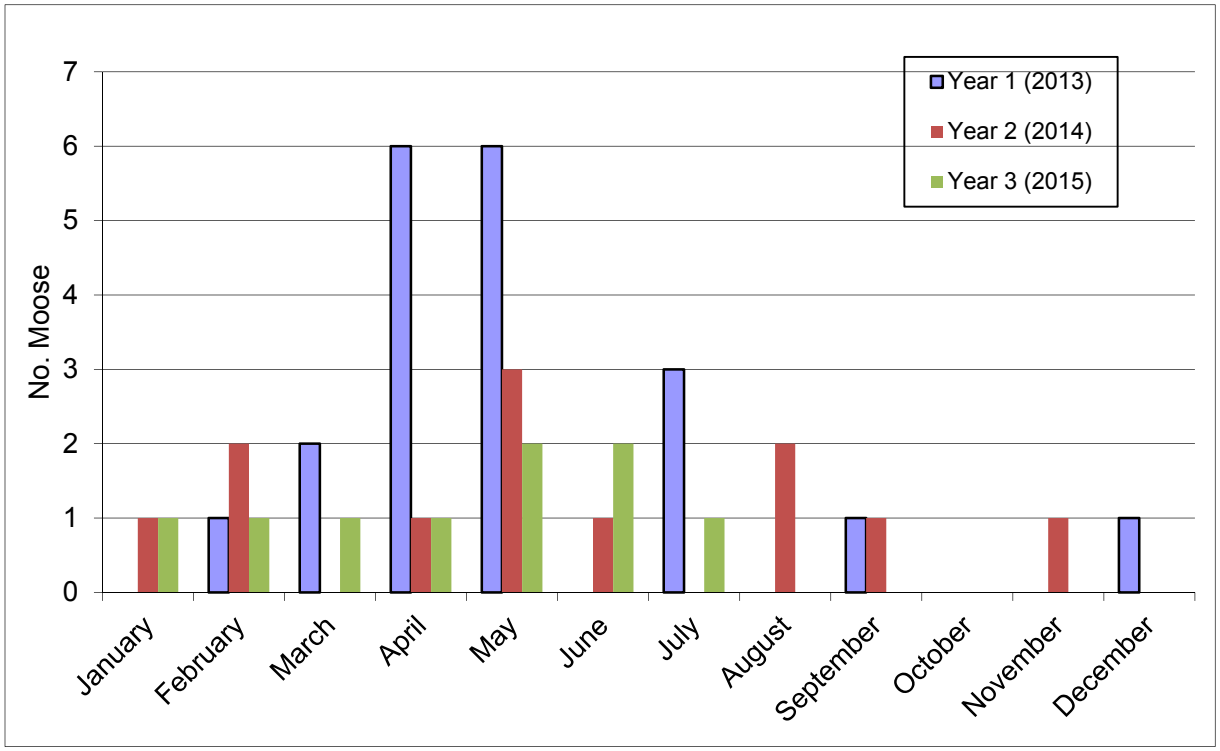

Figure 3. Timing of mortalities for radiocollared, adult moose (*n*=41) from January 2013 through August 2015, northeast Minnesota.

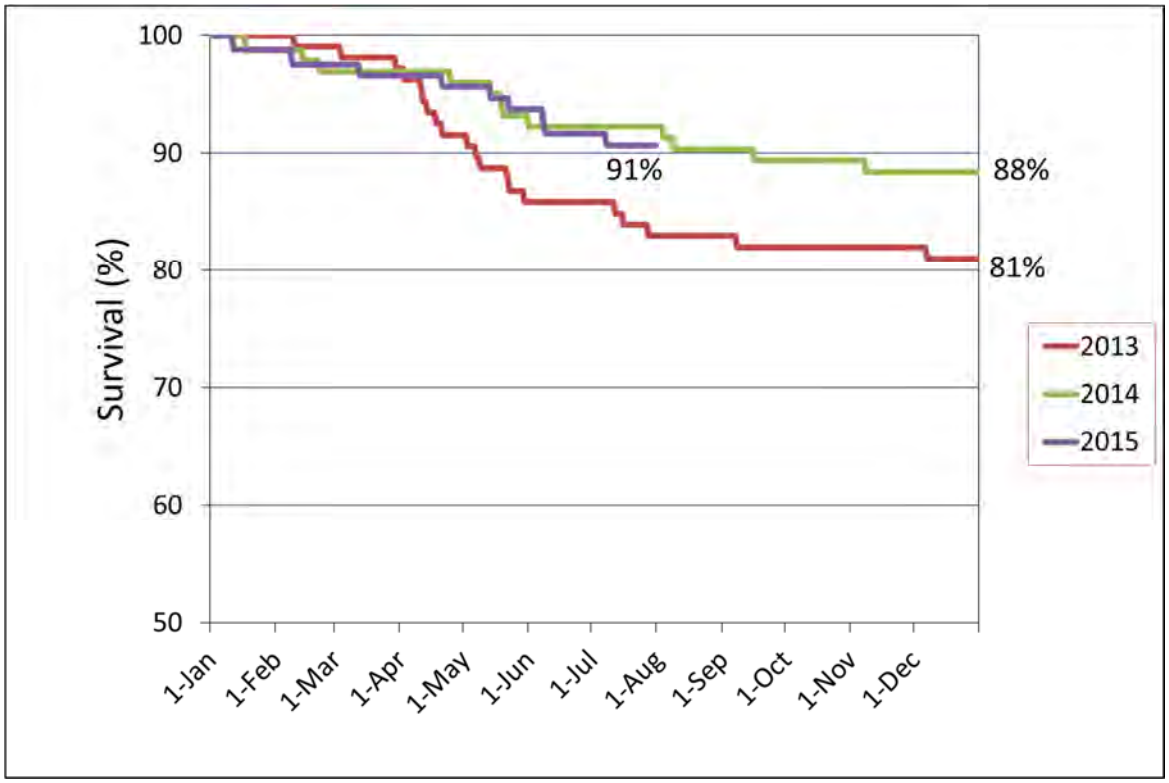

Figure 4. Annual survival of radio-collared, adult moose (*n*=173) captured in 2013, 2014, and 2015 in northeast Minnesota.

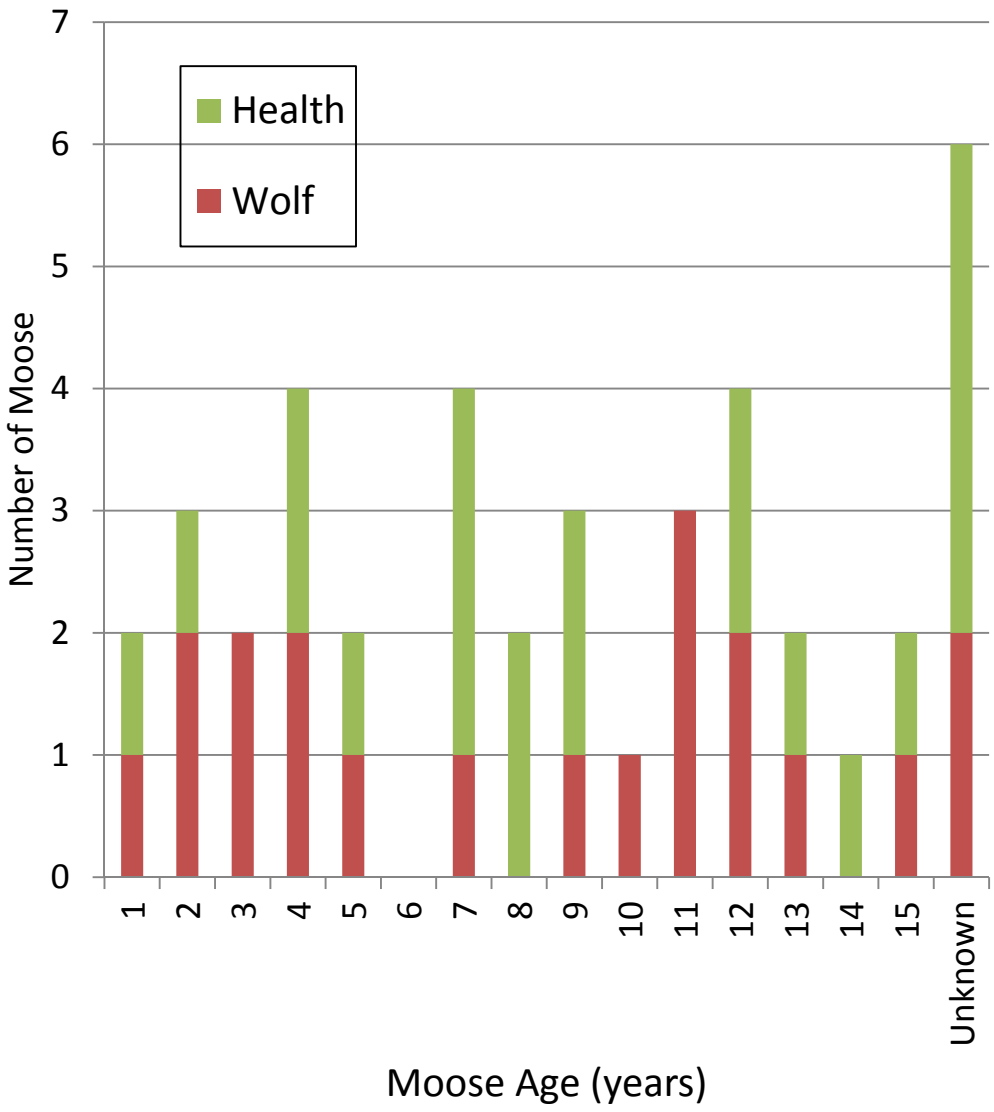

Figure 5. Age of radiocollared, adult moose (*n*=35, 6 moose have ages pending) that died from health-related (green) or wolf-related (red) causes (2013-2015), northeast Minnesota.

MNDNR

HEALTH ASSESSMENT FOR FREE-RANGING ELK IN NORTHWEST MINNESOTA, FROM 2004-2014

Michelle Carstensen, Erik Hildebrand, and Lou Cornicelli

SUMMARY OF FINDINGS

The goal of this project was to assess the health of free-ranging elk (*Cervus elaphus)* from northwestern Minnesota (NW MN) by screening animals for a variety of diseases and parasites. Results indicate which diseases the NW MN elk were exposed to, though not necessarily clinically ill. From the elk (*n*=146) included in this study, our results indicated exposure to eastern equine encephalitis (14%), West Nile virus (64%), malignant catarrhal fever (38%), anaplasmosis (4%), borreliosis (59%), bovine viral diarrhea virus 1 and 2 (10%), bovine herpes virus (5%), *Leptospira sp*., (13%) and parainfluenza virus 3 (30%). A variety of fecal parasites were also identified (*Coccidia, Strongyle-type ova,* and *Moniezia*) in 22% of elk examined. Lung and liver tissue were cultured for bacterial infection; *Streptococcus sp.* was isolated from the lung of one individual and no isolations were found in liver samples. All elk were negative for *Mycobacterium paratuberculosis,* blue tongue virus, neospora, epizootic hemorrhagic disease, brucellosis, chronic wasting disease, and bovine tuberculosis. Hepatic mineral levels were also evaluated.

INTRODUCTION

Elk in Minnesota

Elk (*Cervus elaphus*) are native to Minnesota and were originally distributed across most of the state. They were formally protected from hunting in 1893 and by the early 1900s, over-hunting and prairie conversion to agriculture led to a functional extirpation (Hazard 1982). Reintroduction efforts were initiated in 1914 and 1915 using elk from Yellowstone National Park and Jackson, Wyoming that were translocated to Itasca State Park in north central Minnesota. The herd expanded and in 1935, 27 elk were moved from Itasca State Park to the Red Lake Game Preserve in northwest Minnesota. By the 1940s, the northwest elk population was estimated at nearly 100 animals (MNDNR 2015). Currently, this population (herein referred to as the "Grygla herd") occupies a 45 mi^2 area north of Grygla, Minnesota (Figure 1). Existence of these animals has been controversial and in 1987, the Minnesota Legislature mandated the pre-calving population range between 20–30 animals. Consequently, the Minnesota Department of Natural Resources (MNDNR) instituted elk hunts in 1987, 1996, 1997, and 1998; however, few animals were taken each year (MNDNR 2015). From 2004-2013, hunts have been held annually to keep elk numbers between 30 and 38 animals**.** The 2013, 2014, and 2015 surveys counted 28, 20, and 18 elk, respectively. A second herd of elk occurs in Kittson and Roseau Counties (Figure 2), and is termed the Kittson County herd. These animals were first observed along the Manitoba border in the early 1980s and are loosely segregated into 3 subgroups based on distinctive areas of use (Figure 2). These three subgroups are the Water Tower subgroup (north of Lancaster), the Lancaster subgroup (east of Lancaster) and the Caribou/Vita subgroup (located between Caribou, MN and Vita, Manitoba). The Caribou/Vita herd is known to occupy either side of the international border at any time of year. The extent to which the other two subgroups cross into Canada is unknown. Little is also known regarding the extent of

animal interchange between the Caribou/Vita subgroup and the other two subgroups (MNDNR 2015). Due to crop depredation issues, a hunting season was first held in 2008. The most recent elk survey counted 34 animals in the Kittson County herd. The current Elk Management Plan set a pre-calving population goal for the Watertower and Lancaster subgroups at 20-30 each. The population goal for the Caribou-Vita subgroup is still under discussion with MNDNR and Manitoba Conservation.

Research background

Infectious diseases can reduce reproductive rates and increase mortality and are thus known to regulate wildlife populations (Delahay et al., 2009). For example, meningeal worm (*Parelaphostrongylus tenuis*) has been implicated in both failures to reestablish eastern elk populations and elk population declines in sympatric white-tailed deer range (Raskevitz et al., 1991; McIntosh et al., 2007). Other research has shown that meningeal worm has a minimal long-term impact on elk population growth in Michigan (Bender et al., 2005) or Kentucky (Bolling, 2009). Conversely, research on northwestern Minnesota moose indicated parasites and disease as reasons that population collapsed (Murray et al., 2006).

The objective of our study was to conduct a health assessment of NW MN elk that included: 1) serological survey of pathogens that are known to cause mortality in other mammalian species or are important from a human health perspective; 2) analysis of fecal material for parasites; and 3) examination of tissues to ascertain presence of bacterial infection. Results of this testing could be used to make inferences regarding the potential limiting factors related to elk herd expansion, explanation of disease transfer risk (both zoonotic and wild-domestic), and explanation of potential disease risks to this population.

Further, the discovery of bovine tuberculosis (TB) in cattle and free-ranging deer from 2004-2009 has brought increased scrutiny as to the health status of the NW MN elk, particularly the Grygla Herd. While overlap in range between elk and known TB-infected deer or cattle farms is known to occur, there has been no evidence of TB-infection in MN's elk herd. TB-infected cattle and deer in MN shared the same strain, which is considered of Mexican or southwest US origin, and was not related to the strain of bovine TB found in elk in Manitoba's Riding Mountain National Park. Although Minnesota is now declared 'bovine TB' free, interest remains regarding the role elk may play in future disease maintenance and/or transmission.

METHODS

For this report, all elk sampled from NW MN were grouped as either *harvested* animals (including hunter-harvested, removed under shooting permit, and illegally poached) or *other* (including vehicle kills, sick, and found dead elk). All elk within the harvested category were assumed to be representative of healthy individuals within the population.

Hunters were asked to collect samples of lung, liver, feces, blood, hair, ticks, and an incisor for aging from hunter-harvested elk. MNDNR provided a project overview, instructions of sample collection, and sampling kits at the mandatory elk hunter orientation sessions. Elk shot through depredation permits or other methods were sampled by trained MNDNR. For sick animals, displaying clinical signs of illness, every effort was made to obtain intact carcasses for full necropsy at the University of Minnesota Veterinary Diagnostics Laboratory (VDL), St. Paul, MN.

All equipment needed for hunter-harvested sampling was included in the sampling kit: soft-sided cooler; 1-60cc syringe for blood collection; 6-15cc serum tubes for blood storage; 3 whirlpaks for a sample of liver, lung, and feces; 2 specimen jars with formalin for liver and lung samples; 2 coin envelopes for hair and tooth; datasheet; protocol; Sharpie marker; 1 pair of large vinyl gloves; and 1 ice pack. Successful hunters dropped off their sampling kits when they registered their animal and also provided information on the location of their kill.

Hunters collected blood from the chest cavity as soon after death as possible, using a 60 cc syringe. The blood was placed in serum tubes and kept cool until they were delivered to official MNDNR registration station. Liver and lung samples were collected and split, with half

placed in a formalin jar, while the other half was frozen in whirlpak bags. If the hunter found anything unusual, such as a large abscess or tumor, those samples were also collected and split between the preservation methods (formalin fixation and freezing). Complete sets of samples were not collected from all elk included in this project, as field conditions and sample quality varied; however, there were very few errors in tissue identification or insufficient sample quantities in those submitted. Blood was centrifuged at the registration stations and serum was extracted and frozen. Cranial lymph nodes and obexes were removed by trained MNDNR staff at the registration stations to allow for chronic wasting and bovine tuberculosis testing. Where appropriate, MNDNR made arrangements with taxidermists to collect samples from trophy animals. All samples were submitted to the VDL, where the majority of the testing occurred; some tests were outsourced to the National Veterinary Services Laboratories (NVSL) in Ames, IA. Teeth were sent to Matson's Laboratory (Milltown, MT) for aging by cementum annuli.

RESULTS AND DISCUSSION

A total of 146 elk (83 females, 63 males) were included in this health assessment project (Figure 3). Harvested elk accounted for 138 of the animals (106 hunter-harvested, 29 shooting permits, and 3 poached). In addition, 8 other animals were sampled (2 roadkill, 3 found dead, 3 clinically ill (observed with neurological symptoms) elk that were euthanized by gunshot. Necropsy results from 2 of the clinically ill elk confirmed migrations tracks from *P. tenuis* infection; insufficient samples were collected from the third sick elk to diagnose any illness. Exact age was determined for 130 elk (*M* = 4.4 years; *SE*= 0.3 years; range 0.5 to 16 years old (Figure 4).

Serologic results from harvested elk indicate exposure to eastern equine encephalitis, West Nile Virus, malignant catarrhal fever, anaplasmosis, borreliosis, bovine viral diarrhea virus 1 and 2, bovine herpes virus 1, *Leptospira* spp., and parainfluenza virus 3 (Table 1). Liver samples from harvested elk were evaluated for heavy metal and mineral status (Table 2). Fecal samples from 114 elk were screened for evidence of parasites. Parasites were identified in 25 samples (21.9%), including *Fascioloides magna (n=10), Coccidia sp. (n*=2*), Strongyle-type ova (n*=8*), Moniezia sp (n*=2*), Capillaria sp. (n*=1*),* and mite ova *(n*=2*).* Negative results do not necessarily mean the animal was parasite-free, only that it was not actively shedding at the time the feces were collected.

Our testing indicated that elk in NW MN were not exposed to brucellosis, blue tongue virus, epizootic hemorrhagic disease, neospora, mycobacterium paratuberculosis, bovine tuberculosis, or chronic wasting disease (Table 1).

Mosquito-borne diseases

Positive results were reported for 14 (14.4%) and 36 (37.5%) elk tested for eastern equine encephalitis (EEE) and West Nile Virus (WNV), respectively (Table 1). Both of these arboviruses are spread by mosquitoes, with EEE typically posing a greater mortality treat to most species. Clinical signs of EEE in horses and sheep involve neurologic signs and often death (Bauer et al. 2005, Rutledge 2008). It is also a zoonotic disease and human infections are reported to the Center for Disease Control. Schmitt et al. (2007) reported clinical infection of EEE in free-ranging white-tailed deer (*Odocoileus virginianus*) in Michigan, suggesting this disease can cause mortality in wild cervids and maybe often be overlooked if biologists are only seeking to rule-out chronic wasting disease (CWD).

Little is known about the effects of WNV in elk. Palmer at al. (2004) reported WNV infection in 2 reindeer (*Rangifer tarandus)*, which was the first confirmed cases of this disease in cervids. A wild white-tailed deer in Georgia was reported to die from a WNV infection (Miller et al., 2005). As with EEE, clinical signs of WNV include ataxia, tremors, head tilt, and depression; which are commonly reported neurological signs in wild cervids with numerous causes (e.g., brain abscess, CWD, blunt trauma, etc.); thus, true WNV infection may be under-reported.

Tick-borne diseases

Positive results were reported for 58 (59.1%) and 4 (3.8%) elk tested for *borrelia burgdorferi* (Lyme disease) and anaplasmosis, respectively (Table 1). Borreliosis is a tick-borne (*Ixodes sp*.) bacterial disease that is maintained through sylvatic cycles involving a variety of species, including mammals (primarily wild rodents as the reservoir hosts) and birds. Clinical disease typically includes arthritis and neurologic or cardia dysfunction. While evidence of natural infection exists in wild cervids through serosurveys, there has been no documentation of clinical disease in elk.

Anaplasmosis (*Anaplasma phagocytopila*, formerly *Ehrlichia phagocytophila*) infection in sheep and cattle produces significant effects on the immunological defense system, increasing their susceptibility to disease and secondary infections (Larsen et al., 1994). Experimental studies have shown that elk can harbor asymptomatic infections with *A. marginale* and *A. ovis,* the causes of anaplasmosis in cattle and sheep, respectively. Renshaw et al. (1979) experimentally inoculated elk with *A. marginale* from infected cattle and these elk produced disease in spenectomized bovine calves. This study suggested that free-ranging elk could become infected and act as a reservoir for this disease, but wouldn't likely compromised their survival. This implication of elk as a disease reservoir for anaplasmosis could undermine elk population expansion in NW MN, as cattle producers in the area have been concerned with disease transmission risks between at the wildlife-cattle interface. However, efforts to recover *Anaplasma* spp. from free-ranging elk populations have been unsuccessful, suggesting that even though these species are susceptible, they are probably not responsible for maintaining infections or acting as a source of infection for cattle (Corn et al., 2001). Clinical anaplasmosis has not been reported in elk.

Malignant Catarrhal Fever

A total of 36 (37.5%) elk were positive for peroxidase-linked assay (PLA) testing for malignant catarrhal fever (MCF) in this study (Table 1). Virus neutralization (VN) testing is performed on all PLA-positive samples; however, all elk were negative on VN. The PLA test is more sensitive than the virus isolation, meaning it is much better at identifying true positives. Whereas, VN is more specific, which means it is better at identifying true negatives. There are a couple of concerns with this testing. First, the PLA reacts with multiple gammaherpes viruses (including strains from wildebeest, sheep and deer). A PLA-positive test does not indicate which strain has been found, only indicated that one of the various strains was detected. The higher the positive value with the PLA test, the stronger the positive in the sample. Second, the VN test only screens for the wildebeest strain (which is exotic to the U.S. and a reportable foreign animal disease) and would be negative if other strains are present. This means a sample that was positive on PLA and negative on VN was likely exposed to a gammaherpes virus, but not the wildebeest strain. We do not know for certain what stain of MCF elk are being exposed to in NW MN.

Gammaherpes viruses have been documented to cause serious illness and death in elk and other ruminants. The clinical symptoms can mimic *Parelaphostrongylus tenuis* infection as the animals often exhibit neurological deficits, blindness, high fever, and salivation. While infection with MCF frequently results in death, carrier status can occur and is identified with serology. Li et al. (1996) found small numbers of United States free-ranging elk were seropositive; these animals were once exposed to MCF viruses but whether they had recovered from a non-lethal disease is unknown. A serosurvey to MCF in Alaskan wildlife reported high antibody prevalences for several wildlife species including 96% in muskox (*Ovibos moschatus*), 95% in Dall sheep (*Ovis dalli*), and 27% in elk; impact on survival is unknown (Zarnke et al. 2002).

Cattle-borne Diseases: Bovine Viral Diarrhea Virus (BVD) 1 and 2, Bovine Herpes Virus 1 (BHV), and Parainfluenza Virus 3 (PI)

Positive results were reported for 11 (10.1%) and 5 (4.5%) of elk tested for BVD and BHV, respectively (Table 1). BVD is considered a major disease of cattle and is thought to be

the most common infectious cause of reproductive failure in beef herds in the western U.S. BVD also causes enteritis, mucosal disease, infections, and respiratory disorders in cattle, though experimentally inoculated non-pregnant elk showed no clinical signs and remained healthy for >50 days post inoculation (Barber-Meyer et al, 2007). Tessaro et al. (1999) demonstrated that while experimentally inoculated elk do not show sign of the disease, they can shed and transmit BVD once exposed. Natural exposure of BVD to wild ungulates suggests a spillover from cattle or maintenance within wildlife populations (Duncan et al. 2008).

Bovine herpes virus type 1 is a disease that can lead to respiratory tract disorders, conjunctivitis, genital disorders and immune suppression. It is believed to infect all ruminant species and has been isolated from a large number of wild species. It is most commonly isolated in feedlot cattle. As with BVD, exposure of elk in NW MN to BHV, both cattle-borne diseases, demonstrates contact between these species (direct or indirect) is sufficient to promote exchange of pathogens.

A total of 31 elk (29.5%) were positive for exposure to parainfluenza virus 3 (Table 1). Domestic ruminants are considered the main source of infection for free-ranging ruminants. PI causes mild respiratory disorders in domestic cattle and sheep that serve as initiators for secondary infections of *Pasteurella* spp., which can result in bacterial pneumonia, but clinical symptoms in wild elk remains unknown (Barber-Meyer et al, 2007).

Leptospirosis

Leptospirosis is a bacterial disease that can infect a wide variety of mammals, both domestic and wild. In ungulates, it causes abortion (Fraser and Mayes, 1986). Exposure usually occurs through direct contact with urine from carrier animals or indirectly by contact with a urine- contaminated environment (Bender and Hall, 1996). Much of the landscape of NW MN contains environments where moist alkaline soils are present to house the bacteria, and it may survive for several weeks (Thorne 1982).

A total of 111 elk were screened for 6 species of *Leptospira,* using a microscopic agglutination test (MAT); 12 elk 10.8%) were exposed to at least one strain, 1 elk was co-infected with 2 strains of *Letospira*.(Table 1). Free-ranging elk in Washington had high seroprevalence to *Leptospira interrogans* and high local productivity, suggesting clinical affects may be more dramatic in cattle (Bender and Hall, 1996).

ACKNOWLEDGMENTS

This project would not have been possible without assistance from a number of MNDNR employees and volunteers. We would like to especially thank staff in NW MN who helped with collecting samples: Joel Huener, Donovan Pietruszewski, Christine Reisz, Randy Pracher, Dawn Plattner, Marshall Deters, Ruth Ann Franke, and Graham Parson.

LITERATURE CITED

Barber-Meyer, S., White, P., and Mech, D. 2007. Survey of selected pathogens and blood parameters of Northern Yellowstone elk: wolf sanitation effect implications. The American Midland Naturalist 158 (2): 369-381.

Bauer, R. W., M. S. Gill, R. P. Poston, and D. Y. Kim. 2005. Naturally occurring Eastern Equine Encephalitis in a Hampshire wether. Journal of Veterinary Diagnostic Investigations 17: 281-285.

Bender L., S. Schmitt, E. Carlson, J. B. Haufler, And D. E. Beyer Jr. 2005. Mortality of Rocky Mountain elk in Michigan due to meningeal worm. Journal of Wildlife Diseases: 41: 134–140.

Bender, L., and Hall, P. 1996. *Leptospira interrogans* exposure in free-ranging elk in Washington. Journal of Wildlife Diseases 32(1): 121-124.

Corn, L. and Nettles, V. 2001. Health protocol for translocation of free-ranging elk. Journal of Wildlife Diseases 37(3): 413-426.

Delahay, R. J., G. C. Smith, And M. R. Hutchings. 2009. The science of wildlife disease management. In Management of disease in wild mammals, R. J. Delahay, G. C. Smith, and M. R. Hutchings (eds.). Springer, Tokyo, Japan. pp 1-8.

Duncan, C., H. V. Campen, S. Soto, I. K. LeVan, L. A. Baeten, and M. W. Miller. 2008. Persistent Bovine viral diarrhea virus infection in wild cervids of Colorado. Journal of Diagnostic Investigations 20:650-653.

Fraser, C. M., and A. Mayes. 1986. Leptospirosis. *In* The Merck veterinary manual. Merck and Company, Rahway, New Jersey, pp. 378-782.

Hazard, E. B. 1982. The mammals of Minnesota. University of Minnesota Press, Minneapolis, MN. 280 pp.

Larsen, H. J. S., G. Overnes, H. Waldeland, and G.M. Johansen. 1994. Immunosuppression in sheep experimentally infected with *Ehrlichia phagocytophila*. Research in Veterinary Science 56: 216-224.

Li, H., D. Shen, D. Jessup, D. Knowles, J. Gorham, T. Thorne, D. O'Toole, and T. Crawford. 1996. Prevalence of antibody to malignant catarrhal fever virus in wild and domestic ruminants by competitive-inhibition ELISA. The Journal of Wildlife Diseases 32(3):437-443.

Mcintosh T, R. Rosatte, D. Campbell, K. Welch, D. Fournier, M. Spinato, And O. Ogunremi. 2007. Evidence of Parelaphostrongylus tenuis infections in free-ranging elk (Cervus elaphus) in southern Ontario. Canadian Veterinary Journal 48: 1146-1154.

Miller, D. L., Z. A. Radi, C. Baldwain, and D. Ingram. 2005. Fatal West Nile Virus infection in a white-tailed deer (*Odocoileus virginianus*). Journal of Wildlife Diseases 41 (1): 246-249.

Minnesota Department of Natural Resources. 2015. Strategic management plan for elk: Division of Fish and Wildlife, Minnesota Department of Natural Resources. 34pp.

Murray, D. L., W. Cox, W. B. Ballard, H. A. Whitlaw, M. S. Lenarz, T. W. Custer, T. Barnett, And T. K. Fuller. 2006. Pathogens, nutritional deficiency, and climate influences on a declining moose population. Wildlife Monographs. 166: 1-30.

Palmer, M. V., W. C. Stoffregen, D. G. Rogers, A. N. Hamir, J. A. Richt, D. D. Pedersen, and W. R. Water. 2004. West Nile virus infection in reindeer (*Rangifer tarandus*). Journal of Veterinary Diagnostic Investigations 16:219-222.

Raskevitz, R. F., A. A. Kocan, And J. H. Shaw. 1991. Gastropod Availability And Habitat Utilization By Wapiti And White-Tailed Deer Sympatric On Range Enzootic For Meningeal Worm. Journal Of Wildlife Diseases 27: 92-101.

Renshaw, H. W., R. A. Magonigle, and H. W. Vaughn. 1979. Evaluation of the anaplasmosis rapid card agglutination test for detecting experimentally-infected elk. Journal of Wildlife Diseases 15(3):379-386.

Rutledge, C. R. 2008. Eastern Equine Encephalits *in* J. L. Caprinera, editor, Encyclopedia of Entomology, 1265-1268, Springer Netherlands.

Schmitt, M., S, Cooley, T., Fitzgerald, S., Bolin, S., Lim, A., Schaefer, S., Kiupel, M., Maes, R., Hogle, S., and D. O'Brien. 2007. An outbreak of eastern equine encephalitis virus in free-ranging white-tailed deer in Michigan. Journal of Wildlife Diseases 43(4): 635-644.

Tessaro, S., P. S. Carman, and D. Deregt. 1999. Viremia and virus shedding in elk infected with Type 1 and virulent Type 2 bovine viral diarrhea virus. Journal of Wildlife Diseases 35(4):671-677.

Thorne, E. T. 1982. Leptospirosis. *In* Diseases of wildlife in Wyoming. E. T. Thorne, N. Kingston, W. R. Jolley, and R. C. Bergstrom (eds.). Special Publications Section, Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 46-52.

Zarnke, R. L., H. Li, and T. B. Crawford. 2002. Serum antibody prevalence of malignant catarrhal fever viruses in seven wildlife species from Alaska. Journal of Wildlife Diseases 38(3):500-504.

Table 1. Serological results from harvested elk in northwestern Minnesota, 2004 2014.

Disease	*n*	Apparent prevalence %
Eastern Equine Encephalitis	97	14.4 (*n*=14)
Malignant Catarrhal Fever	96	37.5 (*n*=36)
West Nile Virus	89	64.1 (*n*=57)
Anaplasmosis	104	3.8 *(n=4)*
Borreliosis	98	59.1 (*n*=58)
Brucellosis	112	0
Bovine Viral Diarrhea Virus 1 and 2	109	10.1 (*n*=11)
Bovine Herpes Virus	111	4.5 (*n*=5)
Blue Tongue Virus	112	0
Epizootic Hemorrhagic Disease	112	0
Leptospira Bratislava	111	0.9 (*n=1)*
Leptospira Canicola	111	0
Leptospira Grippothyphosa	111	0
Leptospira Hardjo	111	0.9 (*n*=1)
Leptospira Interrogans Serovar Icterohaemorrhagicae	111	9.1 (*n*=10)
Leptospira Pomona	111	1.8 (*n*=2)
Neospora	104	0
Parainfluenza Virus 3	105	29.5 (*n*=31)
Mycobacterium paratuberculosis	99	0
Bovine Tuberculosis	113	0
Chronic Wasting Disease	115	0

Table 2. Hepatic mineral values of harvested elk in northwestern Minnesota, 2004-2014.

Element (units)	*n*	Mean	Standard deviation	Minimum	Maximum
Arsenic (ppm)[1]	122	0	0	0	0
Boron (ppm)	72	0.148	0.04	0.12	0.21
Barium (ppm)	72	0.14	0.03	0.12	0.17
Calcium (ppm)	72	51.73	16.49	27.6	111
Cadmium (ppm)	122	0.23	0.16	0.06	0.82
Cobalt (ppm)	122	0.07	0.02	0.03	0.13
Chromium (ppm)	72	0.32	0.04	0.32	0.32
Copper (ppm)	122	15.01	14.68	1.3	87.1
Iron (ppm)	122	173	135	37.9	946.3
Mercury (ppm)[2]	81	0	0	0	0
Potassium (ppm)	72	2631.86	226.48	1934	3031
Magnesium (ppm)	113	164.25	20.33	86	214
Manganese (ppm)	116	2.62	0.99	0.28	5.60
Molybdenum (ppm)	122	1.10	0.34	0.39	1.77
Sodium (ppm)	72	897.51	190.27	579	1490
Phosphorous (ppm)	72	4190.17	579.40	1650	5354
Lead (ppm)	122	1.42	0.37	0.04	3.49
Antimony (ppm)[3]	72	0	0	0	0
Selenium (ppm)	122	0.94	0.42	0.25	1.90
Thallium (ppm)[4]	81	0	0	0	0
Zinc (ppm)	122	25.35	9.57	13	89

[1]Cut-off values for arsenic ranged from <0.03 to <0.50 (ppm); all elk were below these thresholds.

[2]Cut-off values for mercury ranged from <0.13 to <.2.0 (ppm); all elk were below these thresholds.

[3]Cut-off values for antimony were <1.0 (ppm); all elk were below these thresholds.

[4]Cut-off values for thallium ranged from <0.03 to <2.50 (ppm); all elk were below these thresholds.

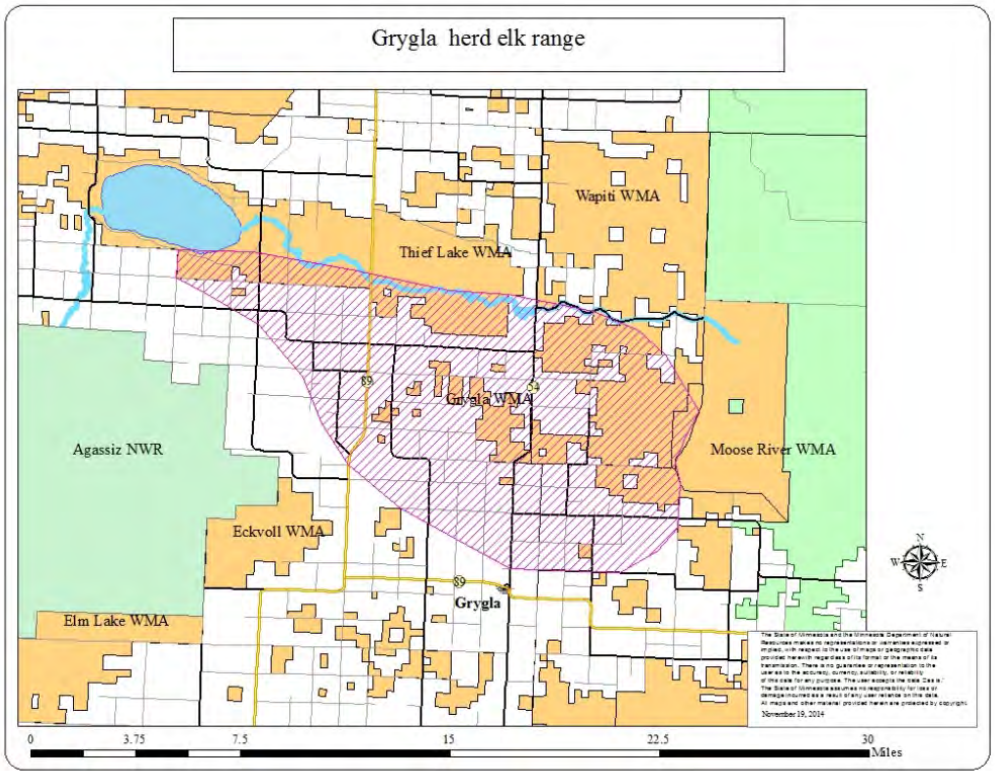

Figure 1. The Grygla elk herd, a remnant from a 1935 reintroduction effort, primarily occupies a 45 mi^2 area north of Grygla, Minnesota.

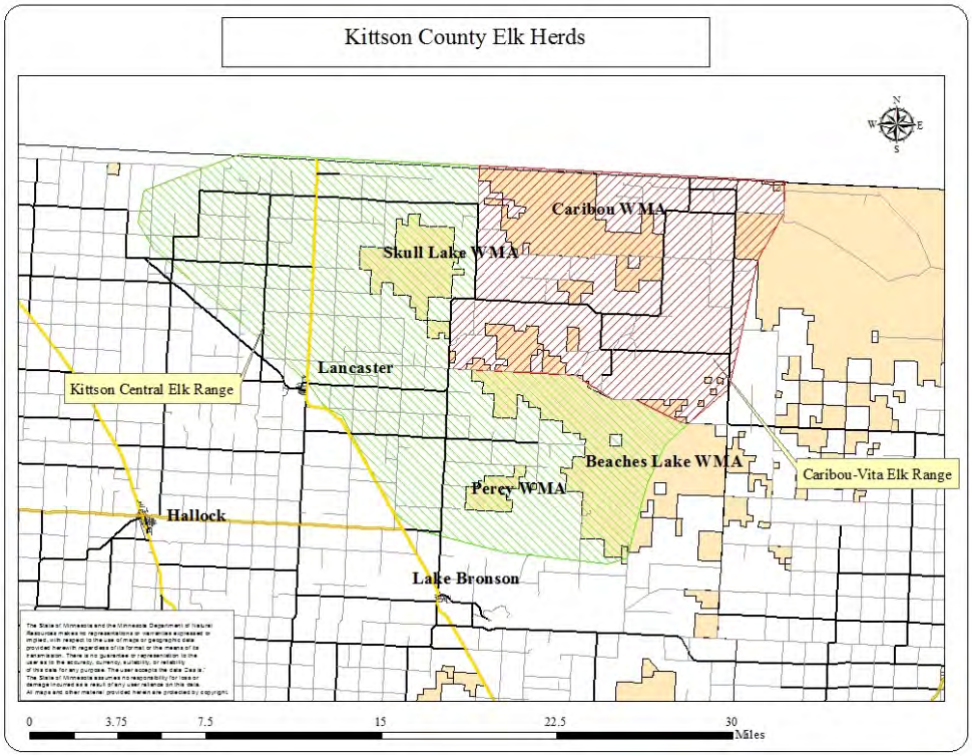

Figure 2. Kittson County elk range.

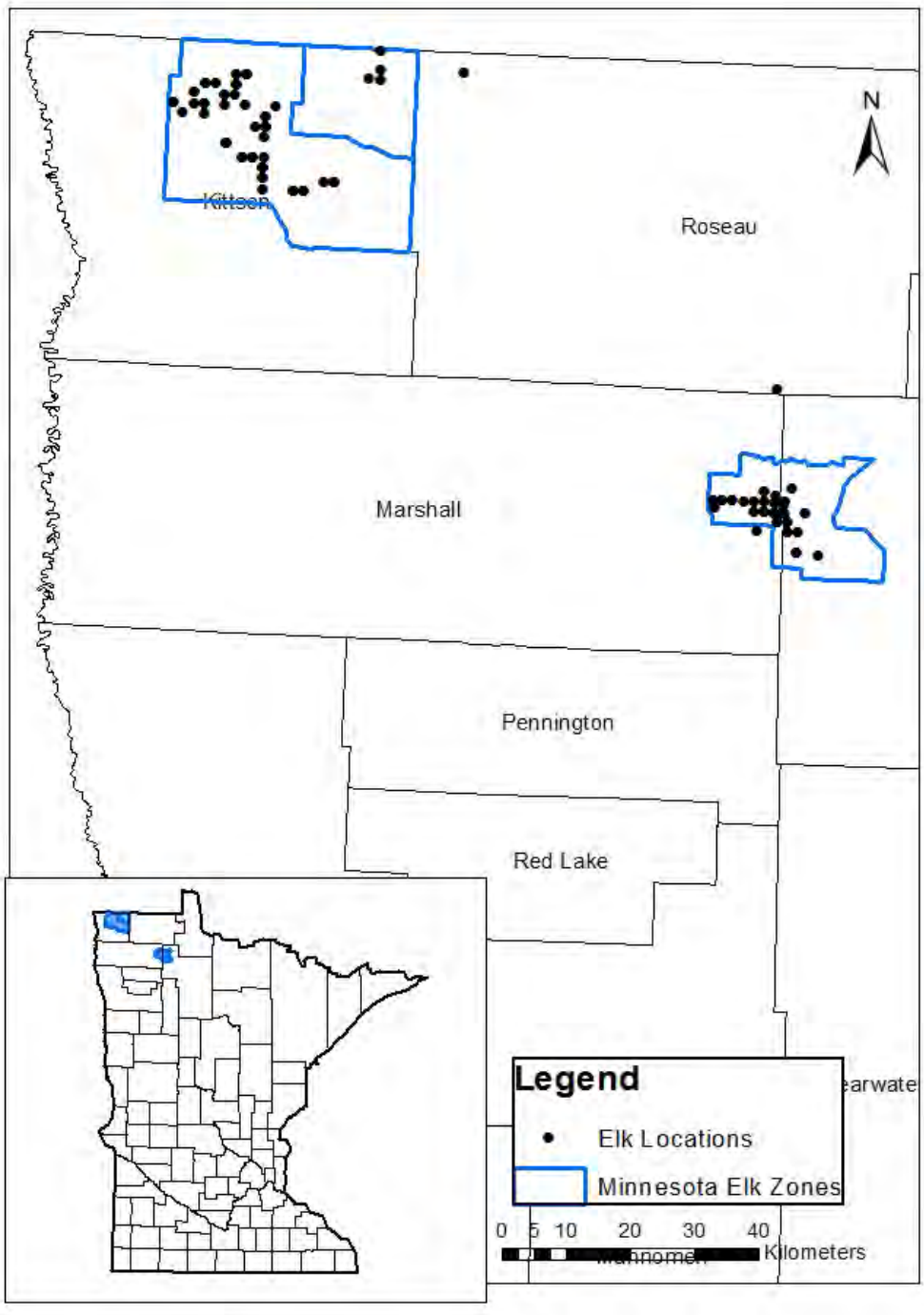

Figure 3. Locations were elk (*n*=146) were sampled for health status in northwest Minnesota, 2004-2014.

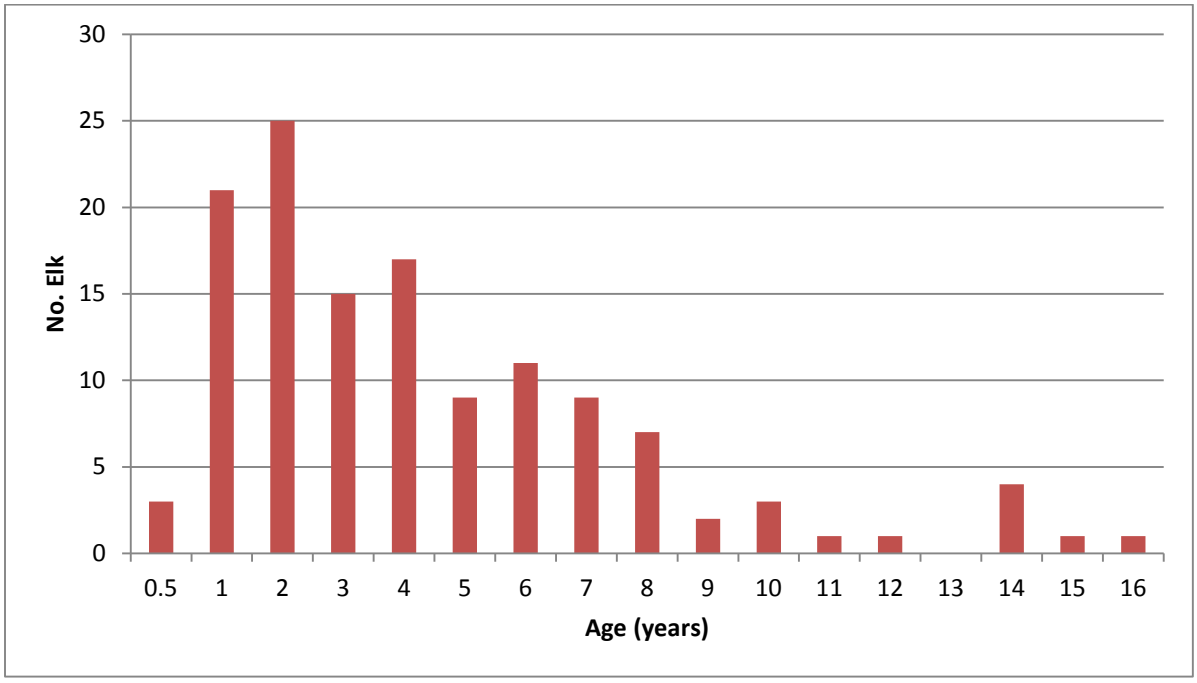

Figure 4. Age distribution of elk (*n*=130) included in the 2004-2014 health assessment project, northwestern Minnesota.

SEROPREVALENCE, ISOLATION, FIRST GENETIC CHARACTERIZATION OF *TOXOPLASMA GONDII*, AND CONGENITAL TRANSMISSION IN WILD MOOSE FROM MINNESOTA, USA[1]

Shiv K. Verma[2], Michelle Carstensen[3], Rafael Calero-Bernal[2], Seth Moore[4], Tiantian Jiang[5], Chunlei Su[5], Jitender P. Dubey[2]

ABSTRACT

Toxoplasma gondii infections are widespread in white tailed deer (*Odocoileus virginianus*) but little is known of its prevalence in other cervids in the USA. Moose (*Alces alces*) is a popular large game animal, hunted for its meat and trophy antlers. Here, we report seroprevalence, isolation and genetic characterization of *T. gondii* from moose from Minnesota. Antibodies against *T. gondii* were detected in 8 of 79 (10%) moose tested by the modified agglutination test (MAT 1:25 or higher). The myocardium of 68 moose was bioassayed individually in mice, irrespective of serological status. *T. gondii* was detected in 3 moose (2 adults, 1 three-week old). The parasite from 2 adults was further propagated in cell culture. PCR-RFLP genotyping of cell culture derived tachyzoites using 10 genetic markers, SAG1, SAG2 (5'and 3' SAG2, and alt.SAG2) SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico revealed two different ToxoDB PCR-RFLP genotypes (#5, designated TgMooseUS1, and #7, TgMooseUS2). The mice inoculated with myocardium of the juvenile moose developed antibodies against *T. gondii* and DNA extracted from infected mouse brain was only partially characterized by PCR-RFLP genotyping, which suggests a potential new genotype. Result documented prevalence of *T. gondii* in moose, and its possible transplacental/transmammary transmission of *T. gondii* in moose.

[1] Parasitology Research, 2015, *In Press*

[2]United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD, 20705-2350, USA

[3]Minnesota Department of Natural Resource, Division of Wildlife, 5463-C West Broadway, Forest Lake, MN, 55056, USA

[4]Biology and Environment Grand Portage Band of Chippewa, 27 Store Rd., Grand Portage, MN 55605, USA

[5]Department of Microbiology, University of Tennessee, Knoxville, Tennessee, 37996-0845, USA

HABITAT FUNCTIONAL RESPONSE MITIGATES REDUCED FORAGING OPPORTUNITY: IMPLICATIONS FOR ANIMAL FITNESS AND SPACE USE[1]

Garrett M. Street[2], John Fieberg[2], Arthur R. Rodgers[3], Michelle Carstensen[4], Ron Moen[5], Seth A. Moore[6], Steve K. Windels[7], and James D. Forester[2]

ABSTRACT

Animals must selectively use landscapes to meet their energetic needs. Depending on an animal's state, it may use a critical resource more or less frequently, and trade-offs involving the use of different habitats may depend on their availability and environmental conditions (e.g., temperature). For example, habitat selection at high temperatures may favor areas that provide thermal cover at the cost of reduced foraging efficiency under consistently thermally stressful conditions. We estimated individual step selection functions (SSFs) with shrinkage using telemetry data from 134 adult female moose (*Alces alces*) in Minnesota, U.S.A., and 64 in Ontario, Canada, to assess the consistency of habitat selection with variation in temperature, time of day, and habitat availability. We averaged model coefficients across all animals within a site to quantify selection strength for five habitat types differing in forage availability and thermal cover. Moose in Ontario favored areas dominated by deciduous and mixedwood forest, consistent with selection for foraging habitat across both the diurnal cycle and a wide range of temperatures. Space-use and habitat-selection patterns exhibited by moose in Minnesota were more dynamic and were indicative of time- and temperature-dependent trade-offs between use of critical foraging habitat (i.e., deciduous) and thermal cover (i.e., conifer, treed wetlands). The amount of deciduous forest associated with both used and available locations declined during mid-day and also with increasing temperatures. Yet, the rate of decline was higher for available than used points, indicating a scale-dependent functional response in habitat selection driven by the trade-off between selection for foraging habitat and thermal cover. These trends suggest that variation in landscape composition and quality interact to produce complex patterns of space use and habitat selection, and foraging animals exposed to increasingly high temperatures may mitigate fitness losses due to reduced foraging efficiency by increasing their selection for foraging habitat in sub-prime foraging landscapes.

---

[1] Journal of Animal Ecology, 2015, *In Review*

[2]Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota-Twin Cities, 2003 Upper Buford Circle, St. Paul, MN, 55117, USA

[3]Centre for Northern Forest Ecosystem Research, Ontario Ministry of Natural Resources and Forestry, 955 Oliver Road, Thunder Bay, ON, P7B 5E1, Canada

[4]Division of Fish and Wildlife, Minnesota Department of Natural Resources, 5463-C West Broadway, Forest Lake, MN, 55025, USA

[5]Natural Resources Institute, University of Minnesota-Duluth, 5013 Miller Trunk Highway, Duluth, MN, 55811, USA

[6]Grand Portage Band of Lake Superior Chippewa, 27 Store Road, Grand Portage, MN, 55605, USA

[7]Voyageurs National Park, National Park Service, 360 Highway 11 East, International Falls, MN, 56649, USA

DESCRIPTIVE EPIDEMIOLOGY AND WHOLE GENOME SEQUENCING ANALYSIS FOR AN OUTBREAK OF BOVINE TUBERCULOSIS IN BEEF CATTLE AND WHITE-TAILED DEER IN NORTHWESTERN MINNESOTA[1]

Linda Glaser[2], Michelle Carstensen[3], Sherry Shaw[4], Suelee Robbe-Austerman[5], Arno Wunschmann[6], Dan Grear[7], Tod Stuber[7]

ABSTRACT

Bovine tuberculosis (bTB) was discovered in Minnesota through routine slaughter surveillance in 2005 and the resulting epidemiological investigation led to the discovery of infection in both cattle and white-tailed deer in the state. From 2005 through 2009, a total of 12 beef cattle herds and 27 free-ranging white-tailed deer (*Odocoileus virginianus*) were found infected in a small geographic region of northwestern Minnesota. Genotyping of isolates determined both cattle and deer shared the same strain of bTB, and it was similar to types found in southwest United States and Mexico. Whole genomic sequencing confirmed the introduction of this bTB into Minnesota was recent, with little genetic divergence. Aggressive surveillance and management efforts in both cattle and deer continued from 2010-2012; no additional infections were discovered. Over 10,000 deer were tested and 705 whole herd cattle tests performed in the investigation of this outbreak.

[1] PLOS ONE, 2015, *In Review*

[2]Minnesota Board of Animal Health, St. Paul, Minnesota, United States of America

[3]Wildlife Health Program, Minnesota Department of Natural Resources, Forest Lake, Minnesota, United States of America

[4]Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Madison, Wisconsin, United States of America

[5] National Veterinary Services Laboratories, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Ames, Iowa, United States of America

[6]Department of Veterinary Population Medicine, Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, United States of America

[7]Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Fort Collins, Colorado, United States of America

MOSQUITOES IN MOOSE COUNTRY: POTENTIAL ARBOVIRUS VECTORS IN NORTHERN MINNESOTA[1]

A.C. Kinsley[2], R.D. Moon[3], K. Johnson[4], M. Carstensen[5], D. Neitzel[6], and M.E. Craft[1]

ABSTRACT

A mosquito surveillance study was conducted following the discovery of serologic evidence of eastern equine encephalitis virus (EEEV) and West Nile virus (WNV) in moose and elk in northern Minnesota. Adult mosquitoes were collected at twelve sites using carbon dioxide traps throughout the summer of 2012. Specimens were counted, identified to species, sorted into pools, and tested for EEEV and WNV. None of the pools were positive for either virus. Low numbers of *Culiseta melanura* (Coquillet) (Diptera: Culicidae) and greater numbers of previously identified eastern equine encephalitis virus and West Nile virus vectors were present in both study regions. Mosquito vectors for arboviruses historically present in Minnesota, such as La Crosse virus and western equine encephalitis virus, were also collected in the study locations. Our findings extend the known range of *Culiseta melanura, Anopheles barberi* (Coquillet) (Diptera: Culicidae), and *Anopheles quadrimaculatus* (Say) (Diptera: Culicidae) into regions of Minnesota with evidence of wild ungulate exposure to eastern equine encephalitis virus, and document the presence and abundance of twenty seven other mosquito taxa in the region.

[1] Journal of the Mosquito Control Association, 2015, *In Review*

[2]Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, Minnesota, United States of America

[3]Department of Entomology, University of Minnesota, Saint Paul, Minnesota, United States of America

[4]Metropolitan Mosquito Control District, Saint Paul, Minnesota, United States of America

[5]Minnesota Department of Natural Resources, Wildlife Health Program, Forest Lake, Minnesota, United States of America

[6]Minnesota Department of Health, Saint Paul, Minnesota, United States of America